VITAMINS

IN THEORY & PRACTICE

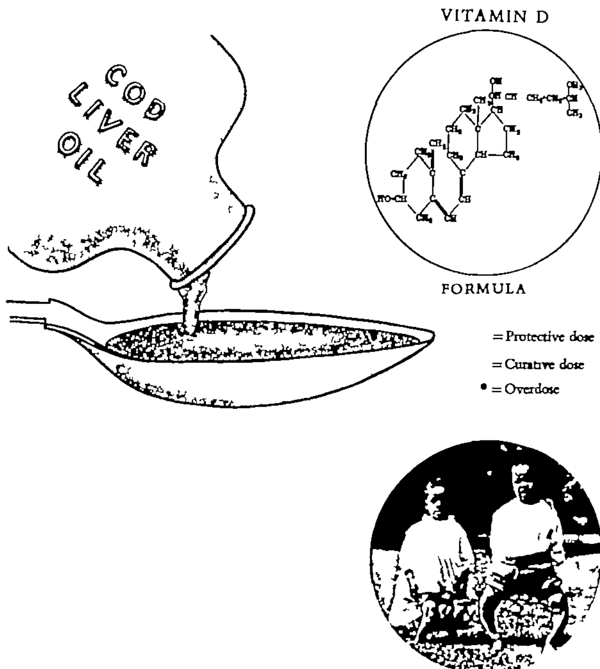


Fig 1 The scarcely visible amount of vitamin D present in a daily dose of cod-liver oil and (insets) its chemical structure and the effects of its shortage

The daily doses are shown actual size. Cod-liver oil, although one of the most potent natural sources, contains only 1 part in 400,000 of vitamin D so that 1 tablespoonful contains about $\frac{1}{38}$ of a milligramme. In ordinary foodstuffs the quantity is considerably less. Thus 100 quarts of milk contain no more vitamin D than about the size of a pin's head.

(There are several forms of vitamin D and the formula given is that for D_3 the kind present in fish-liver oils See p 178)

VITAMINS
IN THEORY & PRACTICE

BY

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Medical Research Council*

FOURTH EDITION

With 103 illustrations, 107 tables



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Der Mensch ist, was er isst

BY THE SAME AUTHOR

Vitamins and Vitamin Deficiencies Vol. I 1938

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Hopkins and Biochemistry (with E. Baldwin and others) 1949

CONTENTS

LIST OF ILLUSTRATIONS

PREFACE

This little book forms the subject-matter of a series of four Thursday afternoon lectures given at the Royal Institution London in 1934. It is published in response to the request of many of those who attended these lectures and of others interested in the fascinating topics with which I have had to deal.

It will be readily understood that it has not been my aim, nor has space permitted me, to give any detailed technical discussion of various highly specialized aspects of the science of vitaminics My purpose has been rather to paint in the high lights. I have tried to present a readable narrative of that truly romantic subject, the history of vitamin discovery and research, which I hope may be intelligible and of interest to the general reader If I have not succeeded in this the blame is certainly with me and not with the opportunities the subject offers. At the same time a conscientious attempt has been made to include some brief mention at least of all the more important findings of modern work, so as to furnish a sort of summary of our present knowledge It is hoped, therefore, that the book may be useful to many professionally interested for example to science students medical men household economists and social welfare workers University Extension pupils and others.

* * * * * * *

As mentioned elsewhere, a number of research workers consider it wrong to engage in popular or semi-popular science writing Two arguments may be urged in reply First, it is an unfortunate fact that at present many if not most members of the general public and sometimes even of the medical profession derive their knowledge of vitamins largely from the *ex parte* advertisements of patent remedy proprietors or their commercial travellers. Secondly books and articles written by those without special knowledge and experience tend too frequently to err in fact as well as in emphasis and it is hard to see why the demand of the public for knowledge should be refused.

The origin of the present book will, it is hoped, be found sufficient explanation of its frankly colloquial tone, as well as of the special emphasis given to some of the more recent developments in the subject.

L. J. H.

December 1935

PREFACE TO SECOND EDITION

Research work on vitamins is so intense and progress so fast that one is almost tempted to insert a Stop Press as the easiest means of keeping a book on the subject up to date. The early call for a second edition however gives me in this instance an easy opportunity of making the necessary additions to the text.

Coming to technicalities the following may be noted as among the more significant advances which have been made during the year since the appearance of the first edition the chemical characterization of vitamin B_1 (see p 56) the isolation of vitamin E (p 244) and the recognition that vitamin B_2 is a complex consisting of at least three factors (p 34)

L. J H.

December 1936

PREFACE TO THIRD EDITION

The text has again been brought up to date. Among some of the more important events of the past year has been the following the synthesis of vitamin B_1 (p 57) and further discoveries as to its mode of action (p 69) the indication of a connection between nicotinic acid (or amide) and the pellagra-preventing vitamin (or pro-vitamin) (p 87) additional proof of the distinction between vitamins D_2 and D_3 (p 178) and the isolation of vitamin A in a crystalline condition (p 221)

L J H

February 1938

PREFACE TO FOURTH EDITION

The first three editions of this book appeared in the space of just over two years Then came the Second World War and the issue of the fourth edition already due, had to be postponed both because of the paper shortage and because the author was occupied with more pressing matters

After the war the work of revision and re revision could be put in hand once again But, so vast was the amount of fresh knowledge that had accumulated in the intervening years that the task of incorporating all the necessary additional material has entailed considerably more labour than did the writing of the whole of the original book itself—and indeed has taken several years to complete. The publishers have been understanding long-suffering and forbearing

In this edition will be found four entirely new chapters All the remainder of the text has been revised, too and brought up to date, paragraph by paragraph and line by line There are 37 additional illustrations and 64 additional tables and appendices and—for the more chemically minded reader—98 formulae of vitamins, in their various forms and of related substances. All told a round score of vitamins are discussed in this edition as compared with the bare half-dozen known when the first edition appeared. Perhaps the most outstanding among the newer ones are vitamin K (the blood-clotting vitamin) and vitamin B_{12} (the anti-anaemia factor)

The aim, as in earlier editions has been to include, in language intelligible to the lay reader some mention at least of every known fact of importance about the vitamins

L. J H.

Summer 1954

The A B C of Vitamins

A

Oh fine and fat was Ralph the rat,
   And his eye was a clear cold grey
How mournful that he ate less fat
   As day succeeded day
Till he found each cornea daily hornier
   Lacking its vitamin A
      I missed my vitamin A, my dears
         That rat was heard to say
      And you'll find your eyes will keratinize
         If you miss your vitamin A

B

Now polished rice is extremely nice
   At a high suburban tea,
But Arbuthnot Lane remarks with pain
   That it lacks all vitamin B
And beri-beri is very very
   Hard on the nerves, says he
      Oh take your vitamin B my dears!
         I heard that surgeon say
      If I hadn't been fed on standard bread,
         I shouldn't be here today

C

The scurvy flew through the schooner's crew
   As they sailed on an Arctic sea.
They were far from land and their food was canned
   So they got no vitamin C.
For 'Devil's the use of orange juice'
   The skipper 'ad said, said he.
      They were victualled with pickled pork, my dears,

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  The skipper 'ad said, said he
    They were victualled with pickled pork, my dears,

Those manners bold and free,
Yet life s but brief on the best corned beef
If you don t get vitamin C

D

The epiphyses of Jemima s knees
Were a truly appalling sight
For the rickets strikes whom it jolly well likes
If the vitamin D s not right,
Though its plots we foil with our cod-liver oil
Or our ultra violet light.
So swallow your cod-liver oil my dears,
And bonny big babes you'll be.
Though it makes you sick it s a cure for the rickets
And teeming with vitamin D

E

Now vitamins D and A, B and C
Will ensure that you re happy and strong
But that s no use you must reproduce
Or the race won t last for long
So vitamin E is the stuff for me
And its praises end my song
We'll double the birth-rate yet, my dears,
If we all eat vitamin E.
We can blast the hopes of Maria Stopes
By taking it with our tea.

C H. A

ACKNOWLEDGEMENTS

mber of colleagues have generously allowed me to reproduce illustra-
from various sources and to them and to their publishers I wish to
ss my great indebtedness

picture of rickets in the frontispiece is from R. H. Major s *The Doctor*
lns (A A. Knopf) the engraving of Lind (Fig 2) from an article by Sir H
ton (*J Roy Navy Med Services* 1915) the crest and photograph of Takaki
3) from the *Japan Ga ette* Peerage of Japan (by courtesy of the British
um) and the photographs of rickets (Fig 4) from E. Mellanby s *Experimental*
s (H.M Stationery Office) and from E Cautley s *Diseases of Children* (Ed.
d & Co) The portrait of Eijkman (Fig 5) was obtained through the kind
es of the Archivist of the University of Utrecht. The picture of the beri-beri
Fig 6) due to A T Stanton is reproduced from the *Studies Inst Med Res*
Malay States 1911 the guinea-pigs with scurvy (Fig 7) are from M. S Rose s
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the *Journal of Physiology* (191) The illustration of beri beri in man (Fig 11)
n Bälz and Miura s *Handb Tr pen Krankheiten* that of polished rice (Fig 12)
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n pigeons (Fig 14) from a photograph by Professor Drummond in Plimmer s
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M. S Rose s *Foundati ns of Nutrition* (Macmillan) of crystalline vitamin B_1
17) from an article by Eijkman in *Proc K Akad Wetensch* of pellagra in
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erick and L. Thompson (by courtesy of W B Saunders) and that of crystal-
actoflavin (Fig 84) from Kuhn (*Berichte der deut Chem. Ges* 1933)

e reproductions from Admiral Hawkins (Fig 31) Lind (Fig 32) and Captain
(Fig 33) I obtained through the valued services of the British Museum
ng Room The illustrations of scurvy in adults (Fig 34) are reproduced from
ticle by P Harvier (*Paris Médical* 1917) and from L. Aschoff and W Koch
Epidemiologie des Skorbuts Jena, 1919) of infantile scurvy (Fig 36) from A. F
s *Scurv y Past and Present* (J B Lippincott) of scorbutic guinea-pigs (Fig 7)
E. M Delf (*Biochem J* 1918) the diagram of the symptoms of rickets (Fig 51)
pted from *Harmsworth s Home Doctor* and the X rays of rickets (Fig 53) is from
Huldschinsky s *The Ultra-V iolet Light Treatment of Rickets* (through Messrs

Hanovia, Ltd.) The photograph of osteomalacia (Fig 54) is by R. T Frank from A F Hess's *Rickets Osteomalacia and Tetany* (Kimpton) of juvenile rickets (Fig 55) from L. Findlay in Oliver and Boyd's *Clinical Study and Treatment of Sick Children* (Oliver & Boyd, 1933) and from E. Feer's *Diagnosis of Children's Diseases* (Lippincott) of canine rickets (Figs 56 57) from E. Mellanby's *Medical Research Council Special Report Series*, No 61 (H.M. Stationery Office) and H. Steenbock, in L. B Mendel's *Nutrition* (Oxford University Press and Yale University Press) Messrs Hanovia, Ltd. provided me with the fine photograph of treatment by ultra violet irradiation (Fig 58) and Messrs J Nathan, Ltd. with those of the manufacture of calciferol (Figs 59 60 No 1) The illustration of crystalline vitamin D (Fig 60 No 2) is due to A Windaus (*Verlag Chem*) and the X-ray photographs in Fig. 61 are taken from the *Medical Research Council Special Report Series* No 158 by R. B Bourdillon *et al* (H.M. Stationery Office) The microphotographs in Fig 66 are by Dr J R. M Innes (*Ann Rep., Inst Animal Pathology* Cambridge, 1929–1930) and the photographs of xerophthalmia (Fig 67) from photographs kindly supplied to me by Professor C. E. Bloch The portrait of Livingstone (Fig 68) is from J R. Campbell's *Livingstone* (Benn) the illustrations of xerophthalmia in dogs and rats (Fig 69) by H. Steenbock, E. M. Nelson and E. B Hart, and by L. B Mendel respectively are given by courtesy of the Yale Press. The bust of Hippocrates (Fig 70) in the British Museum is reproduced from C. Singer's *A Short History of Medicine* (Oxford University Press) The section of a keratinized membrane (Fig 74) is due to S B Wolbach and P R. Howe (*Arch. Path Lab Med* 1928 *Am Med Ass*) and the micro-photograph of crystalline carotene (Fig 75) is from L. S Palmer's *Carotenoids* (Chem. Cat. Co) Elementary school children then and now (Figs. 93 94) are from J F C. Haslam's *Recent Advances in Preventive Medicine* (Churchill)

The data on Warrington school children (Fig 95) were kindly sent to me by Dr G W N Joseph, the Medical Officer of Health. The illustrations of the enlarged stomach and intestinal stasis (Fig 101) are due respectively to the Bemax Research Laboratory and to F Hargreaves *et al* (*Trans Roy Soc Canada* 1931)

I am also indebted for permission to reproduce here my own illustrations previously published in the following scientific journals *Biochemical Journal* (Figs 38 39) *Lancet* (Figs 64, 65) *Proceedings of the Royal Society* (Figs. 96–99)

A number of other figures, and many of the tables, have been specially prepared and are given here for the first time.

The A B C of Vitamins by C. H. A. (see p xvii) first appeared in *St Bartholomew's Hospital Journal* January 1928

In this new (fourth) edition many new figures and tables have been included, and grateful acknowledgements are made also to the following: to Mr Meredith Frampton R.A., for permission to reproduce the photograph of his fine Royal Society painting of Sir Frederick Gowland Hopkins (Fig. 9); to E. Bälz & K. Miura for the photographs of polyneuritic symptoms (Fig. 13) from *Handb. Tropen Krankheiten*; to L. S. Fridericia & collaborators, for the chart of a refected rat (Fig. 16) from *J. Hyg., Camb.*, 1927, **27**, 70; to the U.S.A. Department of Commerce for the data from which the pellagra death-rate curve (Fig. 25A) is constructed; to Dame Harriette Chick and colleagues, for the picture of the pellagrous pig (Fig. 29A) from *Biochem. J.*, 1938, **32**, 10; to E. M. Delf & F. M. Tozer for the diagrams of the bone junctions in scurvy (Fig. 35) from *Biochem. J.*, 1918, **12**, 416; to S. Hecht & J. Mandelbaum for the dark adaptation curves (Fig. 73) from *J. Amer. med. Ass.*, 1939, **112**, 1910; to Unicam Instruments (Cambridge) Ltd, for the picture of a photo-electric spectrophotometer (Fig. 78); to my colleagues, A. J. P. Martin & T. Moore for the illustrations of ill-effects seen in vitamin-E deficiency in rats (Figs. 80 and 81) from *J. Hyg. Camb.*, 1939, **39**, 643, as well as for another unpublished photograph of theirs on the same topic (Fig. 82); to H. C. Hou and to V. P. Sydenstricker, respectively, for their photographs of patients with riboflavin deficiency (Figs. 85A, B and C) from *Chin. med. J.*, 1941, **59**, 314 and *J. Clin. Med. (China)*, 1941, **6**, 19, and from *J. Amer. med. Ass.*, 1940, **114**, 2437; to F. Bicknell & F. Prescott, for the microphotograph of crystalline vitamin B_6 (Fig. 86) from *The Vitamins in Medicine* (Heinemann, 1942); to M. Sullivan & J. Nicholls, for the picture of the greying of the fur in pantothenate deficiency (Fig. 87) from *Arch. Derm. Syph.*, 1942, **45**, 917; to H. F. Seibert, and to M. Sullivan & J. Nicholls for the photographs of rats suffering from deficiency of biotin (Fig. 88) taken respectively from Bicknell & Prescott's book, *loc. cit.*, and from *Arch. Derm. Syph.*, 1942, **45**, 295; to R. H. Follis, for the section of the fatty liver in a choline-deficient rat (Fig. 89) from *The Pathology of Nutritional Disease* (C. C. Thomas, 1948); to S. Ansbacher for the grey-haired rat with PABA deficiency (Fig. 90) and to D. W. Woolley for the bald mouse with inositol deficiency (Fig. 91), both from R. H. Follis's book, *loc. cit.*; and to H. T. Dean and his collaborators, for the remarkable chart relating dental caries to fluoride deficiency (Fig. 102) taken from *Publ. Hlth Rep. Wash.*, 1942, **57**, 1155.

I am indebted to my colleague Miss Mamie Olliver for the figures of losses of vitamin C on cooking (Table XLIV) and to T. N. Morris, from whose statistics, given in his book *The Dehydration of Food* (Chapman & Hall, 1947) I have calculated the entries for the saving of shipping space with dehydrated foods (Table XLV).

The picture of pellagra (Fig. 26(4)) is of a case seen by myself and kindly photographed for me by my colleague Professor John Yudkin. Various other

illustrations are taken from papers previously published by myself, or by myself and my colleagues, in various scientific and medical journals, and I am grateful to their Editors for permission to reproduce them here Fig 21 is from *Compt rend., Ve Congrès Int Tech Chim Indust Agric.*, 1937 p 100 Figs 30 41 and 44, respectively are from *Biochem J* 1939 33 2037 (with W D Raymond) 1942, 36 155 (with M. Olliver) and 1942, 36 183 (with L. W Mapson & Y L. Wang) Fig 43 is modified from *Brit. J Nutr* 1947 1 7 (with L. W Mapson) and Figs. 45 47–48 49 and 72, respectively are from *The Lancet* 1943 1 454 (with M. Olliver) 1942, 1 642, 1937 2 181 (with M A Abbasy & P Ellman) and 1937 2, 1009 (with M K. Maitra) The figures in Tables XLVI and XLVII are based on the findings in papers by myself, in *The Lancet* 1942 1 642 and 1943 1 515 respectively

A considerable number of other Figures and Tables are original, and new to this edition.

I cannot sufficiently express my thanks to Miss J E. Etchells for her unremitting (and cheerful) care, skill and efficiency in all the manifold secretarial matters connected with the preparation of this edition.

The structural formulae, and several Figures, have been patiently and accurately drawn, under my supervision, by Mr Alfred Ward.

My friends Mr A. L. Bacharach and Dr S K. Kon were kind enough to read through the page proofs of this edition and I am grateful to them for various helpful suggestions.

Indulgence is asked for any unintentional oversight which may have crept into the above lists.

CHAPTER I

THE DISCOVERY OF THE VITAMINS

The drawing at the beginning of this book conveys, as I hope fairly vividly, some impression of how minute is the amount of a given vitamin present in our food. Without that speck the direst consequences may follow. Provide it, and disease will be prevented (rickets in the example illustrated); or if the disease unfortunately should have already developed, it will be surely cured. Give too much of the vitamin artificially (the largest speck in the drawing) and ill effects may result from its excess—the so-called hyper-vitaminosis.

How have these facts been discovered? How have we been able to find out not only about the actual existence of the vitamin but such things as its chemical composition and even its precise structural formula (i.e. just how the atoms are arranged within its molecule); to isolate it as a pure substance, and to learn something of how it works in the body—the method by which it is able to carry out its vital, seemingly magic, task?

The story forms, as I said in the Preface, one of the most fascinating of romances. Hundreds of scientific workers have been engaged in solving these mysteries, unravelling this most difficult of detective problems. In the course of the following pages I shall try to describe, in I hope relatively non-technical language, how it has been done.

The first thing to discuss is vitamin history. The man-in-the-street likes to associate a single name with each discovery. He says glibly but quite inaccurately, Marconi discovered wireless, Welsbach discovered the incandescent gas mantle, Newton discovered gravitation. Such generalizations may perhaps serve a useful function, as pegs on which to hang the names of discoverers and inventors. Indeed they are almost inevitable in elementary text-books which have to give students facts and proper names suitable for reproduction in examinations. In practice, however, discoveries are not made like that.

What happens almost invariably is that a whole army of workers is engaged in developing the subject Each profits from the experience of the others and carries the matter a little farther in some new direction Knowledge grows in this way by the separate contributions of numerous individuals all added to the common stream To use a political metaphor science nowadays is a *co-operative* affair And of course it is *international* in this co-operation. As J B S Haldane once said, when new scientific knowledge is gained it is not kept for private property but is published for all to use alike

After this rather long digression let us proceed and try to reach some conclusions as to the stages, slow we may be sure which have led to the gradual recognition of the existence of vitamins

THREE DISEASES OF DIET

To begin, we must go back a good way The first step is the realization that certain diseases SCURVY BERI-BERI and RICKETS—now known as vitamin-deficiency diseases—are due to dietary errors (1601 to about 1900) This was discovered long before the word vitamin had been heard of and the full *significance of the discovery was at first not appreciated* at all Not until about 1912 was the real explanation to appear Nevertheless it was the first big step in the long progression of advances leading ultimately to the present position

SCURVY AND ITS CURE

It is some two or three centuries ago since it was discovered that scurvy—the disease of sore gums painful joints and haemorrhages then so familiar to seamen—could be prevented or cured by the inclusion in the diet of green vegetables or quite small quantities of fruit juice. The fuller details I will give later on in Chapter V when discussing what scurvy is and the nature of the anti-scurvy vitamin It is enough at the moment to mention that in 1720 an Austrian army physician, Kramer by name, wrote that 3 or 4 ounces of orange or lime juice will cure this dreadful disease without other help Many others made similar observations The earliest regular use of oranges and lemons or lemon juice, as an antiscorbutic seems to date from 1601 when

they were introduced into the ships of the East India Company at the instigation of the English privateer Sir James Lancaster Lind (1753) in his celebrated treatise on scurvy said that the severest cases could be cured within six days.



Fig 2 Captain Lind, founder of modern naval hygiene and author of the *Treatise on Scurvy* published in 1753

Many earlier writers had already alluded to the apparent value of fresh fruits or vegetables in scurvy but generally in a rather incidental way and the special merit of Lind's book was that he not only reviewed in detail all the past work on the subject but gave for the first time a really convincing account of experimental work (conducted by himself on sailors) establishing beyond doubt the means for curing and preventing the disease by the use of salads, summer fruits, etc.

BERI-BERI AND ITS CURE

This disease marked by wasting and paralysis still so common in the East that many thousands die from it every year was known in China as early as 2600 B C A one-sided diet mainly of polished rice, is the cause. It was a physician in the Japanese navy named Takaki (Director-General of the Navy Medical Service) who in 1882 found that it could be cured by an increase in



Fig 3 Admiral Takaki who banished beri-beri from the Japanese navy

His imperial services were recognized by the Emperor and he was created a Baron. The above photograph and crest are taken from the full account of his Rank and House as given in the *Japan Gazette* Peerage of Japan

the allowance of vegetables fish and meat in the diet and the use of barley in place of rice He did not understand it correctly as a vitamin-deficiency disease but supposed it was due to insufficient protein (i e. meat fish etc.) in the diet But the fact remains that he recognized that it was caused by a badly balanced diet

RICKETS AND ITS CURE

The idea that rickets was due to something wrong with the food was gaining ground in the last quarter of the nineteenth century In a standard text-book on diseases of children published in 1889 we may read The fault is of quality not quantity A child may be reduced to the last stage of atrophy and yet

not be rickety Conversely it may be overfed fat and gross and yet extremely rickety Rickets is produced as certainly by a *rachitic diet* as scurvy by a scorbutic diet (*Artificial Feeding of Infants* Cheadle 1889) Other equally vell known works will be found to contain such statements as Deficiency f fat is the prime cause of the disease and all observers are agreed upon the ttremely beneficial effects of cod-liver oil (*The Nutrition of the Infant*





Fig 4 Rickets, now a preventable disease In the past rickets has been widely prevalent in this country Severe cases, causing lifelong deformity have now become relatively rare. The deformed limbs, pigeon chest and pot-belly are seen in the first picture The close-up illustrates the peculiar bulging forehead. (Photographs after E. Mellanby and E. Cautley)

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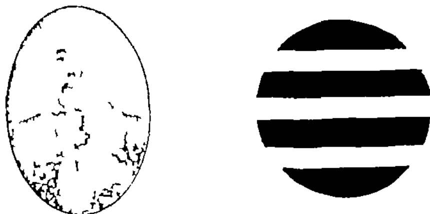


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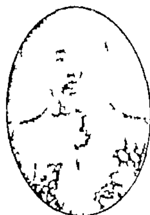




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The idea that rickets was due to something wrong with the food was gaining ground in the last quarter of the nineteenth century In a standard text-book on diseases of children published in 1889 we may read The fault is of quality not quantity A child may be reduced to the last stage of atrophy and yet

not be rickety Conversely it may be overfed, fat and gross and yet extrem rickety Rickets is produced as certainly by a *rachitic diet* as scurvy b scorbutic diet (*Artificial Feeding of Infants* Cheadle, 1889) Other equ well-known works will be found to contain such statements as Deficie of fat is the prime cause of the disease and all observers are agreed upon extremely beneficial effects of cod-liver oil (*The Nutrition of the Inf*





Fig 4. Rickets, now a preventable disease. In the past rickets has been widely prevalen this country Severe cases, causing lifelong deformity have now become relatively r

The deformed limbs pigeon chest and pot-belly are seen in the first picture The close illustrates the peculiar bulging forehead. (Photographs after E. Mellanby and E. Caut

Vincent 1904) The use of cod-liver oil as the perfect cure for rickets indeed been laid down as the accepted teaching in the standard Fre Treatise on Medicine written by A. Trousseau in 1865 English translation which appeared in the U S.A and Great Britain Still earlier in 1838 anot

to prove his point. In 1889 the London surgeon Bland-Sutton u ed cod-liver oil to cure rickets in lion cubs at the London Zoo

We may express the above-mentioned discoveries like this (Table I)

TABLE I *The three dietary deficiency diseases and their cure*

Preserved or salted diets	——→ **Scurvy**
+fresh vegetables or fruit	——→ Scurvy prevented (Lancaster 1601)
Polished rice diets	——→ **Beri-beri**
+increased meat, barley and fruit	——→ Beri-beri prevented (Takaki, 1882)
Diets poor in good fats	——→ **Rickets**
+good fats, cod-liver oil	——→ Rickets prevented

It would be misleading however not to add that at the beginning of the present century much of this teaching was being overlooked, and various alternative theories as to the cause of rickets were being advanced sometimes from authoritative quarters and gained a good deal of support. Such were that it was an infective process or a condition of hypothyroidism or due to confinement and lack of exercise or to lack of lime salts in the food or to excessive production of lactic acid These theories were generally stigmatized in the text-books as being not wholly satisfactory but they died a hard and lingering death. Conclusive *proof* of the dietary origin of rickets was not to be furnished until about 1921 (Chapter VI)

THE PROPHET BUDD

But to resume our story we see that at the beginning of the twentieth century it was clear that two diseases at least, scurvy and beri-beri, and probably a third also rickets could be cured by changes in the diet. Unfortunately the significance of these discoveries was not appreciated, for it was generally supposed that rickets beri-beri and scurvy were due to a germ — some bacterium, or toxin or the like—and not simply to a something missing Perhaps the reason is that it seems easier for the human mind to believe that ill is caused by some positive EVIL AGENCY rather than by any mere ABSENCE of any beneficent property Also the germ theory of disease following on Pasteur s discoveries last century tended to rule all medical thought to the exclusion of everything else.

But in 1840 a medical writer called Budd more far-sighted than his con temporaries seems to have foreseen the existence of the anti-scurvy vitamu —for he definitely expressed his belief that scurvy (to quote his own words) was due to the lack of an essential element which it is hardly too sanguine to state will be discovered by organic chemistry or the experiments of physiologists in a not too distant future His prophecy has been fully justified Perhaps he is lucky in his later glory for after all, he had little or no new evidence of his own for his theory

But the next big step onward is the discovery of experimental beri-beri and scurvy *in animals* It has generally been the rule in medical science, before and since that progress begins and cures for diseases are discovered only when a way has been found of studying the same condition or a similar one experimentally in animals Diabetes is a good example of what I mean

THE DISCOVERY OF EXPERIMENTAL BERI-BERI

It was in 1890 in the Dutch East Indies that Eijkman, a medical investigator sent out by the Dutch Government almost accidentally made the observation which was to lead to the discovery of experimental beri-beri in hens Birds which had been fed on *milled* rice (i.e rice without its bran) so he noticed developed symptoms resembling those of beri-beri. He followed up this clue and in the course of a series of experiments, continued from 1890 to 1897 he was able to show that if the birds were given not milled but *unmilled* rice or rice with the *bran* added they recovered. Eijkman then tried to find out something about the properties of this active curative principle present in rice bran. He discovered among other things that the anti-beri-beri substance could be extracted by water or by alcohol and that it could filter through a parchment membrane (that is it was dialysable) (1897–1906)

Let us make it clear that at first it was not realized that this anti-beri-beri substance was present in and a necessary part of, all normal diets It was regarded rather as a pharmacological antidote or medicine against the beri-beri microbe thought to be present in rice

In 1901 however one of Eijkman s colleagues Grijns suggested for the

first time—and here we have the next small development in the slow march of progress—that beri-beri arose because the diet lacked certain substances of importance in the metabolism of the central nervous system And in 1906 Eijkman himself adopted Grijns s view He said There is present in rice polishings a substance of a different nature from proteins fats or salts which

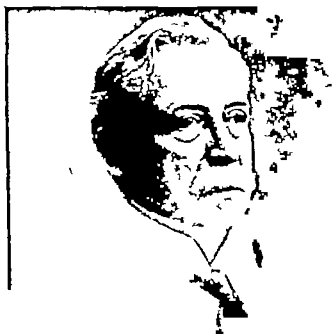


Fig 5 Eijkman, the Dutch biologist, the first to produce a vitamin-deficiency disease in an experimental animal (1897) Awarded Nobel Laureateship jointly with Hopkins (1929)

is indispensable to health and the lack of which causes nutritional polyneuritis

Here then is clear recognition of an anti-beri-beri vitamin, although of course the name itself had not yet been coined.

Following on Eijkman s discovery a certain amount of progress was made in concentrating the anti-beri-beri extracts, and in examining their properties In this connexion one might mention—to show how international was the scope of the research work—such names as Eijkman Grijns Breaudat,

Fraser & Stanton Schaumann Hul hoff Pol Shiga & Kusama Chamberlain & Vedder and others But it was not until the more elaborate attempts at isolation made in 1912 by Funk—the same Funk who introduced the descriptive name vitamin —that really world-wide attention was at last attracted to what we may call the vitamin point of view of beri-beri.



Fig 6 Hen with beri-beri ('polyneuritis') showing the paralysis of its lower limbs.

EXPERIMENTAL SCURVY

After experimental beri-beri experimental scurvy In 1907 two Norwegian scientists Holst & Frolich in Christiania (now Oslo) wanted to produce beri beri experimentally in guinea-pigs, just as Eijkman and others had already got it in birds They happened to be interested in this problem because ship beri-beri was still fairly common among Norwegian seamen To their surprise however the guinea-pigs fed on the unbalanced cereal diets did not develop beri-beri at all but some quite different trouble Holst & Frolich competent observers as they were easily recognized it as scurvy It had all the principal characters of the disease as known in man. Holst & Frolich were acquainted with Eijkman s previous work on beri-beri, and so they straightway recognized that scurvy might likewise be due to some similar dietary deficiency Having thus produced scurvy experimentally they were then able to set to work to investigate the nature of the anti-scurvy factor and in their pioneer investigations they made tests to find out which foods contained it, and how it stood up to heat storage ageing and so forth.

So much then for these three diseases scurvy beri-beri and rickets.





Fig 7 Guinea-pigs with scurvy
Above, normal guinea-pig Below guinea-pigs with scurvy showing characteristic posture (Rose)

THE VITAMINE HYPOTHESIS

The stage is now set for the emergence of the full vitamine hypothesis — the name at this point being spelt vitamine and not as subsequently vitamin

By 191_ a number of facts had been established (1) Beri beri scurvy and perhaps rickets appeared to be due to something missing from the diet. (_) The way had been found of getting *experimental* beri-beri and scurvy in animals (3) Finally work was already in progress on the anti-beri-beri substance One further fact not yet alluded to in our running commentary (but to be discussed fully enough below) had been found by another band of scientists also working on food problems, although from quite another angle We may now put this down as a fourth point in our list (4) This group of workers had come to the conclusion that natural foods such as bread and milk must contain minute amounts of some hitherto unknown substances necessary for life These new substances were of course different from the already recognized and well-known *principal* constituents of food (i.e fat, protein carbohydrate mineral salts water) This fact had first been shown by Lunin as far back as 1888 was confirmed by many others and was finally brought into full publicity by some celebrated experiments published in 1912 by Dr Hopkins (afterwards Sir Frederick Gowland Hopkins and President of the Royal Society) It was in this same year that Funk propounded what he called his Vitamine Hypothesis

TABLE II *The vitamine hypothesis*

Name of disease	Prevented in human beings	In experimental animals	Vitamine hypothesis (Funk, 1912)
Beri-beri	Takak (1882)	Eijkman (1897)	**An anti beri-beri vitamine**
Scurvy	Lancaster Kramer Lind (1601 1720, 1753)	Holst & Frolich (1907)	**An anti-scurvy vitamine**
Rickets	(ca 1900)	—	**An anti-rickets vitamine**

Dr Casimir Funk, a Polish biochemist was working at the Lister Institute in London making a bold effort to isolate Eijkman s anti-beri-beri substance in a pure state. For a time he thought he had got it. He called it the anti-beri-beri vitamine — -amine because he had evidence that it belonged to the group of chemical substances called *amines* vit- because it was so *vital* for life I am informed that this name vitamine was suggested to Funk by Dr Max Nierenstein, Reader in Biochemistry at the University of Bristol



Fig 8 Funk, originator of the vitamine theory (1912).

But the name is not Funk s chief claim to fame Funk advanced the theory that there were four different vitamines — an anti beri-beri vitamine, an anti-scurvy vitamine an anti-pellagra vitamine, and probably also an anti-rickets vitamine Each was responsible for preserving us from its own corresponding deficiency disease—beri-beri scurvy pellagra and rickets respectively

One name in this list, pellagra is new to our discussion, but it is indeed

a vitamin-deficiency disease as surely as are the others and just as important as they and Funk deserves an additional compliment for his sagacious guess

Here then we see the final emergence of the vitamin theory pretty well as we know it to-day—except that to-day it is no longer a theory but a well proved fact

What led Funk to this vitamine theory of his? Merely a close familiarity with, and an intelligent study of the already existing medical literature—not any new discoveries of his own at all. He writes in his paper communicated to the *Journal of State Medicine* (June 1912)

The diseases mentioned above present certain general characters which justify their inclusion in one group called deficiency diseases. They were considered for years either as intoxication by food or as infectious diseases, and twenty years of experimental work were necessary to show that diseases occur which are caused by a deficiency of some essential substance in the food. Although this view is not yet generally accepted, there is now sufficient evidence to convince everybody of its truth, if the trouble be taken to follow step by step the development of our knowledge on this subject. This article is written with the intention of giving a summary of the modern investigations, and by means of a careful selection of references to facilitate the research for anybody who wishes to read the original literature. This careful selection was absolutely necessary for there is perhaps no other subject in medicine where so many contradictory and inexact statements were made, which instead of advancing the research retarded it by leading investigators in a wrong direction.

It seems legitimate to point a moral. The interpreter may be as useful to science as the discoverer. I refer here to any man who is able to take a broad view of what has already been done by others to collect the evidence and discern through it all some common connecting link. At the present day there is a tendency perhaps for the young scientist to think that any small piece of practical work which he can do in a laboratory justifying the publication of a paper is necessarily of greater value than any amount of armchair science —at which he is inclined to sneer

In some matters of detail Funk's views were not quite correct. We know to-day that not all vitamins are amines (that is why we have altered the spelling to vitamin) Nor can we suppose that the anti-beri-beri and anti-scurvy

substances though different are closely related and transformable to a certain extent It only goes to prove that the man who never makes a mistake never makes anything !

We must now break off our consideration of our old friends (I should have said man s old *enemies*) scurvy beri-beri, rickets and apply our attention to that second line of investigation to which we have so far made only brief passing allusion This has to do not with preventing and curing definite diseases but trying to find out what a diet is made up of—its chemical analysis Ultimately these two separate bits of detective work join together for it is found that the same individual parties are responsible in both instances although they had been going under different aliases— vitamins on the one side, and the accessory factors of the diet on the other For the first piece of evidence we must go back as far as 1888

FAILURE OF NUTRITION ON ARTIFICIALLY REFINED DIETS

In that year a pupil of Professor Bunge at Basle, named Lunin, published a paper in which he reported that he had found that laboratory animals (mice in this case) could not survive on synthetic or artificial diets made up of purified fat, protein and carbohydrates plus salts and water that is to say on the known constituents of natural food

This discovery which as we see is now over sixty years old, was a fundamental one But equally important was the deduction which Lunin drew from his results that *a natural food such as milk must therefore contain besides these known principal ingredients small quantities of other and unknown substances essential to life*

Before long Lunin s result had been tested out and duly confirmed by a large number of other investigators mainly in Central Europe. To give a list of these we must include the following names Coppola (1890) Socin (1891) Hall (1896) Hausermann (1897) Falta & Noeggerath (1905) and Jacob (1906) Among these workers Socin in particular expressed very convincingly his belief in the existence of the new unknown substances which, as his

experiments showed were present in egg yolk and milk and which it was the first task of the future to discover Socin like Lunin was a pupil of the celebrated Professor Bunge at Basle and their discoveries should have become widely known since they were mentioned by Bunge in his text-book on physiological chemistry which was the leading one of the time.

The question was approached in a rather different way in Germany by Dr W Stepp but the result was the same He did not use a synthetic diet but tried the effect of subjecting a natural diet such as bread and milk to an extraction process That is to say he devitalized the natural diet by removing this unknown something out of it, and then mice were fed on the extracted residue which remained His mice died on this residue, but when the extract which he had removed from it was put back into it again they flourished. The solvent which he used for washing out this unknown substance from the bread and milk in this way was a mixture of alcohol and ether Stepp could argue from this experiment that bread and milk contains some unknown alcohol or ether-soluble substance indispensable for life

THE IMPLICATION

One fact stands out pretty clearly from all these experiments. The actual amount of these newly demonstrated but so far unidentified substances present in our natural diet (e g in the bread and milk) must be very small We can tell that this is so because chemical analysis shows us that food is made up almost entirely of its *already known constituents* the fat, the protein the carbohydrate the mineral salts and water There is therefore not much room for anything else left over It follows that no matter how essential these newly recognized constituents are they are nevertheless fully potent when taken in quite small amounts A few milligrammes a day make all the difference between life and death. Lunin had certainly realized this and said so specifically But the argument could be put forward even more convincingly after a direct experiment had been specially devised to prove it This was done by a Dutch physiologist, Pekelharing

TABLE III *Experiments on synthetic diets*

Diet	Whether adequate (+) or inadequate (−) for growth and survival
Protein, fat, carbohydrate and salts	−
Milk	+
[LUNIN 1888, SOCIN 1891, MANY OTHERS]	
Bread and milk residue after extraction	−
+ the extracted matter	+
[STEPP 1909]	
Protein, fat, carbohydrate and salts	−
+ a little milk	+
[PEKELHARING 1905]	
Protein, fat, carbohydrate and salts	−
+ 2 c.c. milk, or extract of milk, or yeast	+
[HOPKINS, 1912]	

HOPKINS

The most celebrated experiments on 'synthetic diets' are those of Hopkins published in 1912. It was Hopkins's work, published at the same time as Funk's 'vitamine' theories, which for the first time drew really widespread and general attention to the matter. Needless to say, Hopkins's work was a continuation of that of the earlier investigators, just as every scientific worker must perforce base himself upon the discoveries of his predecessors. This is only to say that each new brick to be laid can rest only on the existing structure. And as so frequently happens, the new development may have been anticipated long previously, but passed over little noticed because the time was not yet ripe for its general recognition. One reason may be that the earlier pioneers had failed to emphasize sufficiently the full significance of their work, and perhaps to give it adequate enough proof. Darwin is counted

THE MAJOR IMPORTANCE OF THE MINOR CONSTITUENTS

Pekelharing first wrote about the result of his experiment in 1905 It had actually been done some years previously Since, unfortunately his report was written in Dutch and since the majority of scientists in the world read only English, German and French, it remained overlooked by most.

What Pekelharing showed was this that a relatively insignificant amount of milk added to the artificial food-mixture of isolated, re-purified fat, protein, carbohydrate, etc. made all the difference, and converted it into a real food, capable of supporting life

This is how Pekelharing described it

Physiology has demonstrated long since that it is impossible to keep an animal alive by feeding it with protein, fat, carbohydrates the necessary salts and water If the food is to have its true value something more than these must be present. What has been published by other investigators I find confirmed in my own experiments.

When white mice are fed on bread baked with casein, albumin, rice-flour lard and a mixture of all the salts which ought to be found in their food, while they are only given water to drink the animals starve to death. During the first few days all is well. The bread is eagerly nibbled and the mice look healthy But soon they get thinner their appetite diminishes and in four weeks all the animals are dead. If however instead of water they are given milk to drink, they keep in good health, though the quantity of albumin, lactose and fat which they assimilate with the milk is quite negligible in comparison with what the bread on which they are fed contains. The element in the milk which keeps the animals alive also occurs in the whey from which casein and fat have been eliminated. Till now my efforts, constantly repeated during the last few years, to separate this substance from the whey and get to know more about it, have not led to a satisfactory result, so I shall not say any more about them My intention is only to point out that there is still an unknown substance in milk, which, even in very small quantities, is of paramount importance to nutrition. If this substance is absent, the organism loses the power properly to assimilate the well-known principal parts of food, the appetite is lost and with apparent abundance the animals die of want. Undoubtedly this substance not only occurs in milk but in all sorts of foodstuffs, both of vegetable and animal origin.

In these last two sentences we see a pretty fair résumé of the vitamin concept as we now know it

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Fig 9 Sir Frederick Gowland Hopkins, O.M., F.R.S (1861–1947)
(After the Royal Society portrait, by Meredith Frampton, R.A.)

In 1929 he was awarded the Nobel Prize in Medicine, jointly with Eijkman, for his experiments on the accessory factors in food. To the professional biochemist his name is known not only in connexion with vitamins, but for pioneer work over a wide range, including the discovery of *tryptophan* and *glutathione* and some classical experiments on *muscle chemistry* Founder of the School of Biochemistry at Cambridge, his shrewd and penetrating mind did much to inspire and stimulate the development of the subject to its present position of importance.

the discoverer of organic evolution not because the conception had never before occurred to another but because his evidence was the first to bring conviction to most of his fellow-scientists

Hopkins's experiments aimed especially at removing various objections which might have been raised against the validity of the conclusion that something else was needed as well as fat protein carbohydrate salts and water Perhaps after all—so it might be argued—the reason why the animals failed to grow on the synthetic diet was because such a regimen was none too palatable or was too monotonous was distasteful or lacked some subtle flavouring substances. So Jacob in Germany and McCollum (a well-known American worker) had supposed But let us examine this suggestion for a moment. On due consideration it does not seem at all plausible after all because we must remember that numerous earlier workers had in fact kept animals for long periods on diets that were nothing if not monotonous For example Socin in Switzerland had maintained his mice in good health for ninety days on a diet of egg-yolk starch and cellulose and Falta & Noeggerath at Basle had supported rats for six months on monotonous diets of milk alone, or milk powder or lean horse meat.

But a particular merit of Hopkins's experiments was that he was anxious to prove beyond doubt that the reason why the rats went downhill on the purified synthetic diet was not simply because they failed to consume enough of it or could not absorb it

We may quote his actual words

The total energy consumption of the animals was carefully determined and it was shown that the rats upon the highly purified dietary ceased to grow at a time when their intake was more than sufficient quantitatively to maintain growth.

On a less pure basal dietary the food consumption necessary for a given weight increment was reduced to one-half or less, compared with that necessary on a pure basal dietary

Cessation of growth upon the pure dietary took place before any failure in appetite although the consumption might later fall to a lower level. Any effects upon the appetite must therefore have been secondary to a more direct effect upon growth processes

Like Pekelharing Hopkins found that the addition of a very small amount of milk to his synthetic diet no more than 2–3 c c. per day (half a teaspoonful) made all the difference between life and death for his rats It can be seen from Fig 10 how with the milk added the rats were able to grow while without it

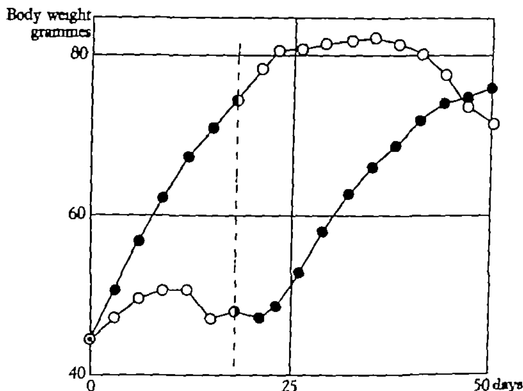


Fig 10 Growth curves of rats, with and without vitamins (Hopkins).
○ Artificial diet alone ● Artificial diet plus milk.

they soon began to lose weight Not only milk, but also extracts derived from milk or yeast he found had a similar effect when given in astonishingly small amounts [1]

[1] According to modern findings 2 c.c. of milk is in fact quite insufficient for optimum growth. Milk is relatively poor in vitamin B_1 so that a very large dose of it is needed every day when the basal diet is made entirely vitamin-free. Although the experimental result is therefore different, the validity of Hopkins's *conclusion* still remains—that the unknown factor is effective in minute doses.

A second point is that later investigation shows that the *amount of food* eaten by the vitamin-deficient rat actually falls off considerably so that in point of sheer *quantity* it is really insufficient and would not do for supporting a normal rat. The deficient rat limits his food intake to roughly the amount he is able to utilize properly which is less than the normal

TWO STREAMS

This takes us near to the end of our historical introduction We see that by 191- enough had been done to prove that vitamins really existed Not much more than their bare existence But the time was now at last ripe for a more general recognition of the fact and one then could begin a bold frontal attack on the harder problem of their intimate nature.

Thus far work had been proceeding along two almost independent channels On the one side it was concerned with showing that animals could not thrive on purified isolated food constituents and with trying to determine the nature of the accessory factors needed (Lunin 1888 to Hopkins 191-) On the other it was devoted to examining the nature of the substances which, it had been found were necessary to prevent certain deficiency diseases The second problem really takes priority chronologically over the first. For we have to recall that since 1753 or earlier it had been known that scurvy could be prevented or cured by additions to the diet that the same was proved for beri-beri in 188-, and was suspected for rickets and that investigations on the nature of the anti-beri-beri substance had already started soon after the discovery of experimental beri-beri by Eijkman in 1890 or on the anti-scurvy substance in 1907-1- (Holst & Frolich)—leading to the final enunciation of Funk s vitamine theory in 1912 The next need clearly was to bring these two sides more closely together In other words what connexion was there between these accessory substances in synthetic diets without which the mice or rats failed to thrive and those other substances in natural food which are needed to prevent scurvy beri-beri, etc ? Hopkins had already seen this implication when in the course of a lecture given before the Society of Public Analysts in 1906 he had dealt with the relations between the analyst and the medical man and remarked in passing

In diseases such as rickets, and particularly in scurvy we have had for long years knowledge of a dietetic factor but though we know how to benefit these conditions

amount in health but what he does eat he makes use of practically as well as the normal animal Again Hopkins s deduction stands good, that failur of appetite is not the *primary* eason for the an mal s llness.

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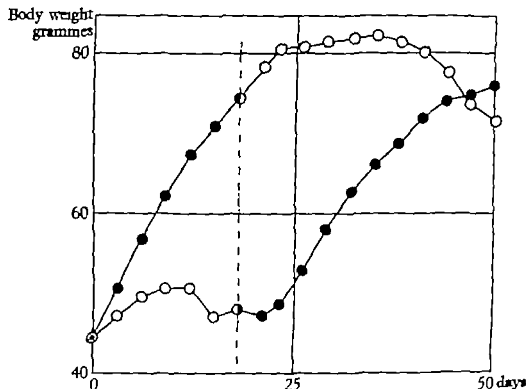


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empirically the real errors in the diet are to this day quite obscure They are how ever certainly of the kind which comprises these minimal qualitative factors that I am considering

It is worth dwelling for a moment on that last sentence Although no more than a single incidental comment made in the course of an address in which many other varied topics also came up in turn for discussion—and although, admittedly it received no general publicity at the time, and so cannot be said to have influenced the general development of the subject—yet here for the first time we see these old observations on deficiency diseases being brought into mental association with those on synthetic diets In this one incidental sentence an unusually penetrating mind had foreseen the important developments which were so soon to follow and indeed had gone some distance towards visualizing the vitamine hypothesis later to be enunciated in a more detailed form by Casimir Funk.

THE END OF ONE PERIOD AND THE BEGINNING OF ANOTHER

But to resume. The year 1912 marked as we have said, the beginning of the new developments and of an increased activity in dietary studies The almost simultaneous publication of the experiments of Hopkins on purified diets and those of Funk on the anti-beri-beri vitamine did much to focus more general attention on the vitamin question and great interest was excited in popular as well as in scientific circles

Hopkins s work got home in a way that the earlier papers of Lunin Socin Pekelharing and the others had failed to do Physiologists were impressed with the exact *quantitative* nature of his experiments and with his anxiety to meet in advance any possible objections

Funk s special service was to crystallize the issue by gathering beri-beri, scurvy rickets and pellagra together under one heading as Vitamine Deficiencies By doing so he created a label for a new department of know ledge Had the separate investigations just continued to go straggling on, each in its own separate way without the new common basis of interest, it is difficult not to believe that progress would have been very much slower

The name itself helped It was vivid and what is more important, it nailed the flag to the mast As Funk has himself confessed I must admit that when I chose the name vitamine I was well aware that these substances might later prove not to be of an amine nature However it was necessary for me to choose a name that would sound well and serve as a catch word (As already mentioned on p 12 some of this credit for the name can go to Funk s colleague and friend Nierenstein) But apart from Funk s theorizing on the vitamine-deficiency diseases there was his own experimental work on the attempted isolation of the anti-beri-beri vitamin Just as Hopkins s investigations were the first to attract general attention to the existence of the unidentified growth-promoting substances so Funk s paper aroused world wide interest in the anti-neuritic (anti-beri-beri) substance. Its extraordinary properties compelled attention A pigeon paralysed and helpless and dying with convulsions could be restored to normal vitality and activity within a matter of minutes when it was dosed with an incredibly small quantity of Funk s vitamin crystals. So small was the actual bulk of the vitamin present in our food and yet it was so indispensable!

MORE THAN ONE ACCESSORY FACTOR

One further step was needed before the vitamin theory was to be finally set up in its modern form This was to show that the unknown something which rats needed to enable them to grow (according to the experiments of Lunin, of Socin, of Pekelharing of Stepp and of Hopkins) consisted of more than one part

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From 1912 to 1915 there was great confusion. At first it was maintained that there was only one unknown substance necessary for growth. Some argued that it was found only in butter and not in yeast, some that it was in yeast but not in butter Some said it was identical with the anti-beri-beri

empirically the real errors in the diet are to this day quite obscure. They are, however certainly of the kind which comprises these minimal qualitative factors that I am considering

It is worth dwelling for a moment on that last sentence. Although no more than a single incidental comment made in the course of an address in which many other varied topics also came up in turn for discussion—and although, admittedly it received no general publicity at the time and so cannot be said to have influenced the general development of the subject—yet here for the first time we see these old observations on deficiency diseases being brought into mental association with those on synthetic diets In this one incidental sentence an unusually penetrating mind had foreseen the important developments which were so soon to follow and indeed had gone some distance towards visualizing the vitamine hypothesis later to be enunciated in a more detailed form by Casimir Funk.

THE END OF ONE PERIOD AND THE BEGINNING OF ANOTHER

But to resume The year 1912 marked, as we have said the beginning of the new developments and of an increased activity in dietary studies The almost simultaneous publication of the experiments of Hopkins on purified diets and those of Funk on the anti-beri-beri vitamine did much to focus more general attention on the vitamin question and great interest was excited in popular as well as in scientific circles

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vitamin, some with the anti-scurvy some said with both some with neither

I do not propose to weary my readers with a detailed history of this period Suffice it to say that the mystery was cleared up in 1915 when two American investigators McCollum & Davis at the University of Wisconsin showed that there were two growth factors at least. The rat was saved by neither alone but needed both. One was found in certain fats e.g in milk-fat (butter) and McCollum & Davis called it the fat-soluble A' factor The other was found in certain watery food-extracts it was soluble in water but not in fats so they called it water-soluble B

TABLE IV

FAT-SOLUBLE A (McCollum & Davis, 1913)

Diet	*Whether adequate (+) or inadequate (−) for growth in rats*
Protein + starch + mineral salts + crude lactose [*contains water-soluble B*]	
+ butter fat *or* **egg yolk extract**	+
+ lard *or* **vegetable oils**	−

WATER-SOLUBLE B (McCollum & Davis, 1915)

Diet	*Whether adequate (+) or inadequate (−) for growth and prevention of polyneuritis in rats*
Protein + mineral salts + butter fat [*contains fat-soluble A*]	
+ wheat *or* **milk** *or* **watery** or **alcoholic extract of wheat embryo** *or* **egg yolk** *or* **ordinary lactose** or **casein**	+
+ repurified lactose or **casein** *or* **polished rice**	−

Fat-soluble A was found in egg-yolk, or olive o
Water-soluble B was fo but not in was also in
and watery or alcoholic ex yolk, and i rt of
unpurified milk-sugar or highly re -sugar or

Soon after it was found that this so-called water-soluble B material not only helped the rats to grow in experiments with these synthetic diets but it also acted as though it contained Funk's anti-beri-beri vitamin i.e. it cured beri-beri Fat-soluble A was later found to have certain vitamin-like properties too that is to say it prevented or cured certain definite deficiency diseases. In other words, the unknown accessory factors of the diet that were needed by the rats to enable them to grow on the synthetic diets were the same as Funk's vitamines that cured and prevented the deficiency diseases

As they were the same why not give them a single name instead of letting them go under two different aliases? This was done Anti-beri-beri vitamine otherwise water-soluble B became known as vitamin B Fat-soluble A became vitamin A Later on we shall discuss the names of the various vitamins, and how many there are of them and how they were distinguished. For the moment we may say that we have now at last completed our task of tracing how the concept of vitamins gradually grew up

THE SCEPTICS

Let us add only this, that it was still to form the subject of rather acute controversy and some disparagement for a few years yet New ideas are not generally popular at first. It needs an effort of thought to get accustomed to them If you have lived in a house all your life and grown to like it to be uprooted is not always welcome and how much more difficult is it to have to change one's whole mental bearings Physiologists had grown so used to calculating dietary values solely in terms of Calories or fuel units, that the uncertainty and complication of *vitamins* was rather distressing As a matter of fact the vitamin protagonists were treated even with a certain amount of derision by their more orthodox opponents But the vitamin protagonists had at least the convincing evidence of their scientific experiments whereas their antagonists generally argued *ex cathedra* To see for oneself the magical effect of a vitamin concentrate when tested on an animal could leave one in no doubt as to the reality of vitamins.

It seems true, to paraphrase William James that there are three stages in the history of every medical discovery When it is first announced people say that it is not true. Then a little later when its truth has been borne in on them so that it can be no longer denied they say that it is not important. After that if its importance becomes sufficiently obvious they say that anyhow it is not new

THE INEVITABILITY OF GRADUALNESS

A former Prime Minister was fond of speaking of the inevitability of gradualness The phrase may be well applied to scientific progress The history of vitamins does illustrate very nicely this gradual process by which new ideas are evolved and new facts brought to light Each investigator in turn gains some hint from the work of others and pushes the argument himself a little farther onward, always making use of, and basing himself upon the earlier discoveries of others The more closely one studies the detailed history of the development of any scientific discovery the more one finds this to be true.

THE COLLECTIVE MIND OF MAN

Ought one not to say therefore that advances in knowledge such as those which we have been discussing in this chapter should be credited to the collective mind of man rather than to any single investigator however notable and brilliant his individual achievement may have been?

At the present time about three separate papers on vitamins are published *every day* throughout the year [1] The task of reviewing and collating this immense output is indeed a laborious one But it is worth while here to emphasize the point that the majority of these thousand papers published each year represent some definite concrete addition to knowledge. Admittedly few of them mark any fresh break-away in the theoretical development of the subject, but nearly all of them do fill in some useful gap it may be in exploring the vitamin reserves of different areas of the globe in discovering new sources of the different vitamins in estimating the prevalence of mortality

[1] The number has increased rather than diminished since this was written for the first edition of this book.

...gating the effect of food treatment on vitamin values ...erent countries or in studying their ...ecting methods of vitamin assay (chemical biological and physical) ...amining the chemistry and the physical chemistry of the vitamins and ...r derivatives in elucidating the physiology of the mode of action of ...amins in the animal economy in applications to medicine so varied as ... of vitamin D in parathyroid tetany for callus formation or in the ...nt of tuberculosis or lupus in applications to agriculture and in ...ning the varying susceptibility and the vitamin needs of many ...species and in many other ways. Much of this work may not be ... but it does at least illustrate my point that many hands are needed ...e pump working from which flows the stream of knowledge.

THE DEVELOPMENT OF IDEAS

...will be said that these conclusions are merely well-worn platitudes ...s in fact commonly recognized that the growth of science is the ...deas rather than the isolated achievements of persons. But ... far this really is recognized. At the recent[1] Faraday celebrations ...peaker told us—it was broadcast for all to hear over the wireless ...ere been no Faraday there would be to-day no radio no tele...graphs no electric trams no motor cars no electric light no ...no electric machinery. Surely this is completely to misunder... of scientific progress as well as Faraday's special achieve...cular claim to fame. His claim is this—that by his penetrating ...y and his experimental brilliance he was able to accomplish ...much as could have been done by perhaps ten of his most ...oraries. But he was nevertheless the child of his age. Others ...he very same problems not conquering them so rapidly it ... of them emerging from his own smaller individual field ...master of all ten. But had Faraday never lived can one ...orable march of science would before long have reached ...Faraday's glory is that he did so much to *hasten* progress

[1] Written in 1934 for the first edition.

Similarly with the vitamin pioneers Two events which specially helped to catalyse the rate of reaction are commemorated by the award of the Nobel Laureateships for 1929 The first was the discovery of experimental vitamin B_1 deficiency by Eijkman in 1897 followed by the first chemical work on the anti-neuritic factor The second was the interest excited by Hopkins s celebrated paper of 1912

IN RETROSPECT

To conclude we have seen that the discovery of vitamins was a gradual process continuing over a period of many years one development slowly merging into another often in almost imperceptible stages Even when the proof for any one position had been won it was not always to receive immediate recognition

The names of the first individuals to reach the more important land marks on the way are all included in our chronological summary in Table V

TABLE V *Chart of vitamin history*

PART I *Deficiency diseases*

In man		
1601	**Scurvy** (Lancaster)	Prevented empirically by dietary additions.
1882.	**Beri-beri** (Takaki)	
1900 *ca.*	**Rickets**	
1847	BUDD	Anti-scorbutic factor postulated.
In experimental animals		
1890–7	EIJKMAN.	Experimental beri-beri discovered. First work on anti-beri-beri factor
1901	GRIJNS.	Beri-beri simply a deficiency disease.
1907–12	HOLST & FRÖLICH.	Experimental scurvy Scurvy similarly a deficiency disease. Work on anti-scurvy factor
1912	FUNK.	The vitamine theory Anti-beri-beri, anti-scurvy anti-rickets and anti-pellagra vitamines postulated.

PART II. *Normal diets*

1888	LUNIN	Purified basal diets inadequate.
1909	STEPP	Extracted bread and milk inadequate
1905	PEKELHARING	*Small* supplement of milk suffices.
1912.	HOPKINS.	Experiments attracting wider attention to these accessory factors.
1915	MCCOLLUM & DAVIS	Two such factors at least.

SUMMARY

We find that dietary deficiencies were long ago shown to be the cause of *scurvy* (1601) *beri-beri* (188-) and *rickets* (late nineteenth century) but the significance of these findings was not fully recognized. In 1897 Eijkman discovered beri-beri in an experimental animal and work then began on the isolation of the anti-beri-beri substance from foods Experimental scurvy was discovered by Holst & Frölich (1907-1-) Funk (191-) propounded the vitamine hypothesis —i e. *scurvy beri-beri rickets and pellagra were each regarded as due to deficiency of separate anti-scorbutic anti-beri-beri anti rickets and anti-pellagra vitamines respectively*

Lunin (1888) followed by Pekelharing (1905) and many others had shown that animals failed to thrive when fed on a purified mixture of the *known* constituents of food so proving that natural foods must therefore contain minute amounts of an additional hitherto unrecognized but essential substance Experiments on such synthetic diets by Hopkins (191-) together with Funk s investigations on the chemistry of the anti-beri-beri vitamin focused general attention on the problem and after that progress became rapid

CHAPTER II

HOW MANY VITAMINS ARE THERE?

A vitamin may be compared in one respect with a chemical element An element has been defined as a substance which *up to the present* has been shown to contain only one kind of matter Similarly with a vitamin What has been thought to be a single vitamin may sometimes turn out to consist really of two or more. When they are split up in this way fresh letters have to be brought up and assigned to the newly discovered constituents—a new water-soluble factor vitamin C is found then vitamin A is split into A and D and then vitamin B into B_1 and B_2 and still later B_2 is itself found to be a complex made up of several different, new vitamins

The chart opposite, which lists some of the more important vitamins now recognized, should help to make things clear (Table VI)

VITAMIN A AND VITAMIN B

We have already learned how McCollum & Davis in America proved that for satisfactory growth rats needed at least *two* different factors (1) fat-soluble A and (2) water-soluble B It was soon noted you will remember that water-soluble B was found in the same foods as the anti-beri-beri vitamine and produced similar effects in animals It came to be assumed therefore that the two were identical and water-soluble B was renamed *vitamine B* Later at the suggestion of Professor J C Drummond of London the terminal *-e* of the word *vitamine* was dropped and it became *vitamin*, to make it quite plain that not all vitamins were necessarily *amines* Nor have they turned out to be, although some of them do indeed contain the amine group in their structure.

Similarly fat-soluble A became *vitamin A* Its absence from the rat's diet was found to give rise to an eye infection, called xerophthalmia and to liability to lung infections and other infective conditions too Osborne &

TABLE VI *Naming the vitamins A list of some of the more important vitamins now recognized*

			Anti-	Formula (and chemical name)
Fat-soluble A → Vitamin A	A		Xerosis	$C_{20}H_{30}O$ (*axerophthol, vitamin A_1*) $C_{40}H_{56}$ (*β-carotene*) (*certain other A vitamins and active carotenoids*)
	D		Rickets	$C_{28}H_{44}O$ (*vitamin D_2 ergocalciferol*) $C_{27}H_{44}O$ (*vitamin D_3 cholecalciferol*) (*several other D vitamins*)
Water-soluble B → Vitamin B	B_1		Beri-beri	$C_{12}H_{17}ON_4SCl\ HCl$ (*thiamine aneurin*)
	B_2	P-P	Pellagra	$C_6H_6ON_2$ (*nicotinamide niacin amide*)
		Riboflavin	Angular stomatitis, cheilosis	$C_{17}H_{20}O_6N_4$ (*riboflavin*)
		Several others	—	*See p* 37
	C		Scurvy	$C_6H_8O_6$ (*ascorbic acid*)
	E		Sterility	$C_{29}H_{50}O_2$ (*α-tocopherol*) $C_{28}H_{48}O_2$ (*β- and γ-tocopherols*) $C_{27}H_{46}O_2$ (*δ-tocopherol*)
	Various others		—	*See p* 37
	K		Hypoprothrombinaemia (causing haemorrhage)	$C_{31}H_{46}O_2$ (*vitamin K_1*), $C_{41}H_{56}O_2$ (*vitamin K_2*) (*naphthoquinone derivatives*) (*several other naphthoquinone derivatives*)

* * *All the above are needed by man and their absence produces well-recognized deficiency diseases (with the possible exception of vitamin E) A supplementary list of additional vitamins is given in Table VII*

Mendel, American workers at the University of Yale were the first to notice this symptom We now know that vitamin-A deficiency in human beings likewise gives rise to these same symptoms—it is common in some parts of the world but not in England

VITAMIN C

We have also to recall from our historical introduction that the pioneer experiments of Holst & Frölich in Norway showed that the scurvy-preventing (anti-scorbutic) factor was different from the anti-beri-beri factor It was found in different foods and also it disappeared more rapidly from them during storage or ageing and in other ways At first the anti-scurvy factor was called water-soluble C then later at Drummond s suggestion *vitamin C*

It was understood that new vitamins when they were discovered should be assigned the succeeding letters in the alphabet, in rotation. Subsequent history has only gone to show how the best laid schemes o mice an men gang aft a-gley

VITAMIN D

For a time it was thought likely that vitamin A might be identical with the anti-rickets vitamin, since it was found that those foods which served to prevent rickets were also generally rich in vitamin A as judged by their power to promote growth in rats or to prevent the eye disease. Professor Edward Mellanby thought the anti-rickets factor was probably the same as vitamin A or at any rate closely associated with it in foodstuffs.

But evidence as to their difference rapidly began to accumulate (1) In the first place it was shown by some American workers (Shipley Park, McCollum & Simmonds 1921) that certain foods which were potent as sources of vitamin A were little good in preventing rickets (2) Later it was found almost simultaneously by various workers both in England and in America that the rickets-preventing factor in cod-liver oil was not destroyed when hot air was blown through it, although this treatment was already known (as shown by Hopkins) to destroy the vitamin A. The two could not therefore be identical (3) Soon methods were obtained of actually separating the anti

rickets factor out of cod-liver oil by an extraction process, leaving the vitamin A behind This was due to an American chemist Zucker and was conclusive evidence (4) Subsequently it was discovered that the anti-rickets factor is formed when ultra violet rays are allowed to play on a waxy constituent of various vegetable tissues called ergosterol and the pure anti-rickets vitamin can be separated from the resulting mixture. This product has no vitamin-A activity Similarly a natural colouring matter found in carrots, butter and other foods, called carotene was shown to have vitamin-A activity but it is not anti-rachitic. Vitamin A a substance chemically related to carotene, was eventually obtained in a virtually pure state and needless to say is entirely distinct from the anti-rickets factor

Since 19-3 the anti-rickets vitamin has been called vitamin D

VITAMIN E

In 1922 Professor H. M. Evans, an anatomist at the University of California at Berkeley discovered a new factor without which rats could not reproduce. It was clearly quite different from vitamins A B C or D for it was not present in diets which had an adequacy of all of these. Other workers in America, named Sure and Mattill reached the same conclusion independently and almost simultaneously

At first the new vitamin was provisionally designated by the letter X or the anti-sterility factor to quote Evans's own words. Later to conform to the regular convention the next letter in the alphabet was taken and the anti-sterility vitamin became vitamin E

VITAMIN B SPLIT INTO B_1 AND B_2

We may recall that Funk in 1912 first suggested that pellagra might be a vitamin-deficiency disease. For quite a decade however pellagra was thought to be caused by an inferior quality of protein. This was due largely to the teaching of Goldberger an American pioneer investigator on pellagra. But by 19-5 Goldberger had satisfied himself that pellagra was indeed a true vitamin deficiency The vitamin in question he showed resembled vitamin B

in some ways since it was generally found in the same foodstuffs. But suspicion had already been growing that vitamin B was quite possibly not a single substance. Goldberger carried out some experiments as a result of which he came to the conclusion that what had been called vitamin B contained at least two vitamins (1) the old anti-beri-beri vitamin and (2) the new anti-pellagra vitamin

Fresh yeast, for example, was effective both in preventing beri-beri and pellagra. But after it had been heated in an autoclave it was no longer of any use in beri-beri although it retained its anti-pellagra potency

Extracts of maize meal on the other hand were rich in anti-beri-beri potency but poor in anti-pellagra. Animal experiments were made to test this. Other workers confirmed Goldberger s findings

Later in the appropriate chapter we shall discuss further distinctions between the anti-beri-beri and anti-pellagra vitamins

Goldberger himself called the two factors the A.-N or anti-neuritic, and P -P or pellagra preventive

Certain American workers, however preferred respectively F (which recalls Funk and his original Vitamine) and G (for Goldberger)—these also being the next two vacant letters. These names, F and G did not have a long life and F was later applied to a totally different vitamin (referred to on p .86) while the label G has fallen into disuse.

In Britain the Accessory Food Factors Committee of the Medical Research Council in 1927 recommended the use of the symbols B_1 and B_2 respectively for the anti-beri-beri vitamin and its newly discovered companion A chief point of contrast between B_1 and B_2 was that B_2 was more heat resisting The name vitamin B_1 was eventually generally accepted for the anti beri-beri factor both in Europe and in America, although for a time in America the term vitamin B was used in this more restricted sense. But the position about vitamin B_2 has become more complicated, for it has been since found that there are several such heat-stable vitamins associated together in yeast and various foodstuffs, and only one of them prevents pellagra i.e. is the true P -P vitamin. Vitamin B_2 as thus defined is therefore a complex including the pellagra-preventing vitamin and several others as well.

THE VITAMIN B_2 COMPLEX

To enter into the full details at least four component vitamins were later recognized in what was at first supposed to be a single vitamin and called vitamin B_2

(1) First we have the P P factor already discussed which prevents pellagra in human beings and a corresponding disease in dogs monkeys and pigs Later in 1937 this vitamin was identified with a substance already known to chemists **nicotinamide**

Three remaining B_2 vitamins which were each for a time confused with the pellagra preventing factor were in turn to be differentiated from it They can be enumerated as follows.

(2) The first is a substance whose existence had been known since 1879 called lactoflavin (since renamed **riboflavin**) but only demonstrated in 1933 to have the nature of a vitamin and to be needed by rats for growth and maintenance of a healthy skin. In its absence human beings also develop certain symptoms which will be discussed in Chapter x.

(3) Next there is a vitamin preventing a so-called *rat pellagra*—really a dermatitis in rats quite distinct from true pellagra. It was at first given the rather unfortunate name vitamin B_6 in 1934, and later renamed adermin, or **pyridoxin**, by which last it is most commonly called. Relatively little is yet known about any practical relation to human nutrition.

(4) Later yet another vitamin was found to be concerned in preventing a disorder in young fowls at first incorrectly called *chick pellagra* Subsequently about 1939 this vitamin turned out to be the same as a certain vitamin-like substance already known to be needed to stimulate the growth of numerous bacteria and other microbes and called **pantothenic acid**

The reader interested in the fuller details can refer to Table VII, and to a later part of the book (Chapter x)

OTHER VITAMINS

For the sake of completeness we must mention that still other B vitamins had earlier been postulated as necessary for rats or pigeons viz. B_3, B_4 B_5 These terms have happily now gone into disuse for they were employed in

rather a vague sense, and we now have instead precise knowledge about the well-defined chemical substances, and the symptoms produced in their absence, as set out in the previous paragraphs

In addition to new vitamins denoted by *letters of the alphabet* (such as F and P) there are yet other vitamins which are described by *chemical names* rather than by alphabetical symbols namely **biotin, choline inositol, *p*-aminobenzoic acid, folic acid strepogenin.** All these are collected together with some explanatory notes, in Table VII and will be mentioned again in Chapters XI and XII. Although all or nearly all of them are perfectly definite substances having a clearly established role for protecting the health of animals under certain conditions we know as yet relatively little about the possible *significance of most of them for human beings* One outstanding exception is **vitamin K** (Table VI and Chapter IX) which has valuable uses in clinical medicine and another is **vitamin B_{12} the anti-pernicious-anaemia factor** (Chapter XI)—the latest addition of all to the family of vitamins and in some ways the most important of them all.

VITAMINS FOR MICROBES

Bacteria and various microscopic forms of life, need substances like vitamins, much as humans and animals do They are described under the general title of growth-promoting factors for micro-organisms Many bacteria also are able to manufacture their own vitamins as they grow If one grows such microbes in broth one can detect the vitamin in the broth after a time by feeding it to a rat. The vitamin in the broth has been synthesized by the micro-organism.

Many of the substances described in the foregoing paragraphs function both as growth-promoting factors for micro-organisms and as vitamins for animals. Some of them such as inositol and *p*-aminobenzoic acid have indeed been studied more in relation to micro-organisms than to animals.

BIOS

Bios was the name given by a Belgian worker Wildiers in 1901 to a substance, or rather a group of substances, of a vitamin-like nature, that stimu-

TABLE VII *List of additional vitamins*

Date when first recognized	Name	Effects of deficiency include	Important clinical interests	Alternative names and descriptions (and formula, if known)
1929	Vitamin F	Scaly tails, etc. in rats	—	Nutritionally essential, unsaturated fatty acids, i.e. (1) linoleic acid ($C_{18}H_{32}O_2$) (2) linolenic acid ($C_{18}H_{30}O_2$) (3) arachidonic acid ($C_{20}H_{32}O_2$)
1933	Pantothenic acid	Dermatitis in chicks	—	Filtrate factor ($C_9H_{17}O_5N$)
1931	Biotin	Seborrhoeic dermatitis in rats	—	Formerly called vitamin H anti-egg white injury factor ($C_{10}H_{16}O_3N_2S$)
1932	Choline	Fatty livers in dogs, etc.	—	($C_5H_{15}O_2N$)
1934	Vitamin B_6	Dermatitis in rats	—	Pyridoxin (formerly called adermin) and derivatives, viz. (1) pyridoxin ($C_8H_{11}O_3N$) (2) pyridoxal ($C_8H_9O_3N$) (3) pyridoxamine ($C_8H_{12}O_2N_2$)
1936	Vitamin P	?Liability to haemorrhage	Doubtful	Citrin including (1) hesperidin ($C_{28}H_{34}O_{15}$) (2) related glycosides
1928, 1940	Inositol	Growth failure in micro-organisms; Baldness in rats and mice	—	Bios I ($C_6H_{12}O_6$)
1940	p-Aminobenzoic acid	Growth failure in micro-organisms; ?Greying of hair in rats, etc.	See next column	p-Aminobenzoic acid ($C_7H_7O_2N$) is the nutrient for micro-organisms which is antagonized by the sulphonamide (= bacteriostatic) class of drugs, now so widely used clinically for combating certain infections
1941	Strepogenin	Growth failure in micro-organisms and (?) in rats, etc.	—	—
1941	Folic acid	Anaemia (macrocytic, megaloblastic)	Cures certain types of anaemia in man	Pteroyl-glutamic acids, e.g. (1) pteroyl-glutamic acid ($C_{19}H_{19}O_6N_7$) (2) pteroyl tri-glutamic acid ($C_{29}H_{33}O_{12}N_9$) (3) pteroyl hepta-glutamic acid ($C_{49}H_{61}O_{24}N_{13}$)
1946	Animal-protein factor(s)	Poor growth in rats, chicks, dogs, monkeys, etc.	—	One such animal-protein factor is vitamin B_{12} (see below)
1947	Vitamin B_{12}	Poor growth in certain micro-organisms; Anaemia (macrocytic, megaloblastic)	Cures pernicious anaemia in man	—

lated the growth of yeast. Under this generic title must now be included three factors inositol (Bios I) pantothenic acid (Bios II A) and biotin or vitamin H (Bios II B) which are also classed as vitamins for animals.

AUXINS

In the vegetable kingdom, accessory factors for growth are also needed by higher plants and these are called auxins

TABLE VIII. *Vitamin-like factors in the plant kingdom*

Growth-promoting factors	Needed by bacteria and other micro-organisms
Bios	Complex of growth-stimulants for yeast
Auxins	Growth hormones needed by higher plants

ISOLATING AND SYNTHESIZING THE VITAMINS

So far we have given little more than a bald account of how each new vitamin came in turn to be recognized as a separate entity or to be differentiated by some characteristic property from one previously known. Simultaneously with this gradual lengthening of the list of the vitamins, work was being pushed forward intensively by hundreds of chemists all over the world, until one by one, each vitamin had been finally separated in a state of purity from some food containing it, and its chemical structure established. Then it could eventually be manufactured synthetically in the chemist's laboratory and given a systematic chemical name Some of this history is summarized in a very condensed form in Table IX. As in later chapters we come to discuss the vitamins individually we shall learn some further details of how all this came about, and how also it has been possible to ascertain the way in which the vitamins exert their function in the body and how their use in medicine has been extended in various ways

That ends our rather long and, I fear bewildering list Perhaps the poem on p xvii which first appeared in a medical students magazine will help to let it sink in—so far as some of the more important letters in the vitamin alphabet are concerned—and with less effort than the foregoing prosy description.

TABLE IX. *Historical chart of progress in vitamin chemistry*

(For brevity and simplicity only four of the best known vitamins are included in the following list)

Deficiency disease	Discovery of action of protective foodstuffs	Date of recognition as a distinct deficiency disease, with a specific protective factor	Date when the vitamin was first isolated (or* alternatively first identified with a previously known substance)	Date when the vitamin was first synthesized (or* alternatively synthesized before it was yet known to be a vitamin)
Scurvy	Fruits and vegetables (1601)	1907	(1932*)	1933
Beri-beri	Whole rice, barley etc. (1882)	1901	1927	1936
Rickets	Good fats (ca. 1900)	1919–22	1932	—†
Pellagra	Good protein foods (1916)	1926	(1937*)	(1867*)

† Vitamin D (or D_2) was prepared artificially by ultra-violet irradiation of the pro-vitamin (ergosterol), as long ago as 1927 but its complete synthesis has still to be accomplished.

CHAPTER III

BERI-BERI AND VITAMIN B_1

It will be remembered that in our historical introduction we were concerned largely with two well-known deficiency diseases namely beri-beri and scurvy the first so common in the East the latter the old disease of mariners These are caused by the lack of the two water-soluble vitamins vitamins B (or B_1 as it is now called) and C We will now turn to a more intimate discussion of these two diseases and their respective vitamins (It seems more logical to take them in this order the order actually in which they were first studied, rather than to run through the vitamins in alphabetical order and by so doing we shall also be keeping the water-soluble vitamins grouped together instead of jumping about from fat-soluble to water-soluble vitamins in turn. There is some convenience in this too because the fat-soluble vitamins are often found together in the same foods and have a number of properties in common, rather marking them off from the water-soluble ones.)

To beri-beri and scurvy we shall also have to add an account of pellagra, the third important disease due to lack of a water-soluble vitamin Pellagra, you will recall is the disease from which thousands of people died year after year in the southern part of the U S A

BERI-BERI AND VITAMIN B_1

Beri-beri, as we have said, is an ailment which is widely prevalent in the East—in the East Indies in Japan in Siam, etc.—especially wherever the staple article of diet is polished rice, that is to say rice highly milled by machinery so that the germ and the outside branny part, both of which contain the vitamin have been removed (Fig 12) The photograph (Fig 11) shows a typical case of dry beri-beri At first the sufferer notices numbness in his legs and later pain in the calf muscles. Finally he becomes exhausted, emaciated and paralysed. He has difficulty in breathing and frequently there is a special kind of heart trouble, namely an enlargement of the right-hand



Fig. 11 An inhabitant of the Dutch East Indies suffering from severe beri-beri (vitamin-B_1 deficiency).

Beri-beri is very prevalent in many zones in the Orient where the diet consists too exclusively of milled rice

side of the heart and an increased rate of beat. Sometimes instead of emaciation there is a great swelling (oedema) in different parts of the body due to the accumulation of water—this variant is called wet beri-beri. If a food containing an adequate supply of vitamin B_1 is given in time the patient *recovers rapidly and suffers no permanent injury Failing this he is likely to die* The immediate cause of death is generally heart failure.

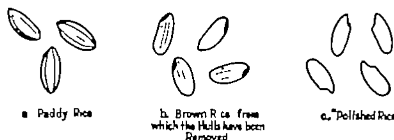


Fig 12 Rice grain. The germ is removed during milling and with it its vitamin B_1

To the physician the most characteristic feature of the disease is the so-called peripheral neuritis (or perhaps more accurately it is a *peripheral neuropathy*) and this is responsible for some of the symptoms, shown in Fig 13 Occasionally the brain too may become affected, and there results what is known as cerebral beri-beri or Wernicke s encephalopathy

FOUR SAILORS IN EVERY TEN ILL WITH BERI-BERI IN THE JAPANESE NAVY

This heading relates to conditions as they used to be, not as they are to-day The banishment of beri beri from the Japanese navy was brought about by Takaki, a name already familiar to us from our historical introduction Takaki s career is of some interest. He entered the Japanese navy as a young medical officer in 1872 Before long he had fully determined that he must find some cure for this dreaded malady beri-beri which wrought such havoc among his men. He decided that his best course in the first place would be to improve his general knowledge of medicine, and he came to London where he was at St Thomas s Hospital for five years. On his return he was





Fig 13 Symptoms of beri-beri.

Above the wrist drop below the contraction of the foot—two of the manifestations of polyneuritis seen in cases of beri-beri.

made Director of the Tokio Naval Hospital, and then he set to work in real earnest on his problem.

In his later reminiscences published in an article in the *Lancet* in 1906 he remarks (somewhat ungrammatically) Such conditions used to strike my heart cold when I came to think of the future of our Empire, because if such a state of health went on without discovering the cause and treatment of beri-beri our navy would be of no use in time of need Incidentally rather a revealing side-light on his motives!

Soon he had satisfied himself that beri-beri must be due to a fault in the diet. After all, the Japanese sailors were as hygienic in their habits as the European, and there seemed no other way of accounting for beri-beri except the difference in the food they ate—largely polished rice He therefore persuaded the Japanese Admiralty to let him make a number of experiments in the navy upon a scale of great magnitude as he expressed it.

EXPERIMENTS IN A TRAINING SHIP

This is how he was able to prove his point A training ship had just returned from a voyage around the world there had been as many as 169 cases of beri-beri, out of its total complement of 376 sailors and officers. No less than twenty-five had died. As Takaki himself describes it The fact was indeed alarming and consequently a careful examination was made. The *Tsukuba* a training ship was then sent out to follow the same course as that the *Rinjio* had taken, with a supply of the food elements the relative proportion of nitrogen to carbon of which was one to fifteen. The result was that there was not a death from disease during the whole voyage

Benefiting from his experience Takaki was able to get an improved diet introduced throughout the navy generally and, within a period of months almost, beri-beri vanished like magic. See Tables X and XI

Takaki still *did not understand the real nature of beri-beri* although he had found a way of preventing it He imagined that it was caused by insufficient protein[1] in the food and too much fat and carbohydrate[2] Looking back, we

[1] Body-building food is the main part of meat, fish, cheese, etc.

[2] Sugar starch and the like produces energy but is not body-building

are now able to say that when he was giving the extra protein he gave also, *without knowing it* extra vitamin B_1 But in other parts of the world little progress was made and thousands still died from beri-beri, which was generally thought to be an infectious disease due to bad sanitation or to a microbe or to contaminated rice

TABLE X *The conquest of beri-beri in the Japanese navy*

	1878	1879	1884	1885	1886	1887	1888
No of cases of beri-beri	1485	1970	718	41	3	0	0
Percentage	33	39	13	0·6	0	0	0

TABLE XI *Number of cases of beri-beri among prisoners in Japanese navy*

	1883	1884	1885
No of cases of beri-beri	69	73	0
Percentage	61	57	0

RICE AND BERI-BERI

The idea that one could prevent beri-beri by giving whole rice instead of polished (or adding the discarded *polishings* to the polished rice) was not to grow up until after Eijkman s experiments on hens, described in Chapter 1 Following on this discovery a colleague of Eijkman in the East Indies was then able to point out by a study of statistics that the prisoners in gaols who developed beri-beri had been fed on polished rice, whereas those who escaped the disease had had unpolished or partly polished rice (Table XII)

TABLE XII. *Beri-beri statistics from prisons in Java*

Diet	No. of prisoners	Cases of beri-beri
White rice	150,266	4201
Partially polished rice	35,082	85
Unpolished rice	96 530	9

In a lunatic asylum an experiment was carried out by Fletcher which proved that beri-beri could be alleviated if steamed rice was used instead of polished rice (Table XIII)

TABLE XIII *A dietary experiment on beri-beri in a lunatic asylum* 1907

Diet	No of patients	Cases of beri-beri	Deaths
Polished rice	1[illegible]0	36	18
Steamed rice	1[illegible]3	[illegible]6	0

Many other instructive examples could be given. Two more will suffice

(1) Beri-beri was stamped out in an American army corps in the Philippines by the use of unpolished instead of polished rice (Table XIV)

TABLE XIV *An experiment on beri-beri in a U S Army Corps (Philippine Scouts)*

Date	Diet	Cases of beri-beri
1909	Potatoes, beef and white flour + **polished rice**	618
1911	+ **unpolished rice and beans**	3
1913	+ **unpolished rice and beans**	0

(2) Before 1914, 900 cases of beri-beri were seen every year in children in Manila. Ninety-five out of every hundred of those ill, died from it. In 1918, when the use of rice polishings as an antidote had become universal, the disease had disappeared

I hope at any rate that I have said enough to convince you that whenever polished rice has been the principal article of diet and beri beri has resulted, the substitution of unpolished rice for the polished has always vanquished the disease.

BERI-BERI PREVENTED BY BROWN BREAD

But it is a mistake to think that beri-beri is seen only in rice-eating populations It may occur on any badly balanced diet containing insufficient vitamin B_1 e g on a one-sided diet containing little else but white bread In such cases the simple substitution of brown or wholemeal bread for white bread will prevent it

(1) For example in a Danish whaling expedition (reported by Nissen in 1930) in the Southern Seas fifty-one cases of beri-beri occurred on one ship on which no wholemeal bread was eaten On five other ships in the same fleet but provided with wholemeal bread and a diet otherwise similar no beri-beri was seen

(2) In a prison in Elizabeth New Jersey white bread was for a time used as the staple article of a very limited dietary Beri-beri broke out among the prisoners Brown bread put an end to the trouble

(3) And here is an instructive story of *How a shipwreck prevented beri-beri* In the northern areas of Labrador and Newfoundland where many of the inhabitants subsist on a diet of white bread, molasses and salt meat, cases of beri-beri have long been common. In 1910 a ship ran ashore, laden with a cargo of wholemeal wheat-flour and in order to lighten her a considerable portion of her load was removed and was subsequently consumed by the people in the adjacent districts. The result was that no case of beri-beri was reported in that region for a period of one year following this event. (Little, quoted by Ellis & Macleod.)

BERI BERI AND RELIGION

At the Siege of Kut (1916) British troops with a ration of white flour biscuits and fresh horse meat developed beri-beri Their Indian comrades, on the other hand whose ration comprised barley plus preparations of whole wheat grain remained free from beri-beri but since their religious scruples prevented their eating the meat (which contains the anti-scurvy vitamin) they fell victims to scurvy instead!

These few instances show how the too exclusive use of *white bread* may lead to beri-beri Here is one final and truly remarkable, example of beri-beri, noted by Dr G W Bray

Inebriety v beri-beri and the League of Nations A certain Pacific island, Nauru in Polynesia, is an Australian mandated territory and there the aborigines were in the habit of consuming an alcoholic beverage prepared from yeast The League of Nations stepped in and prohibited its use. The result was that inebriety disappeared among the adults only to be replaced by outbreaks of beri-beri among the breast-fed infants. The forbidden beverage had been their mainstay against beri-beri. Faced with the new dilemma the authorities thereupon permitted the reintroduction of the toddy but with the proviso that it was not to ferment for long Promptly the infant death-rate fell from 50 to 7 per cent White flour and polished rice are now prohibited on this island and only wholemeal flour and brown rice may be purchased in the native stores

BERI-BERI IN RECENT YEARS

Beri-beri is still prevalent in rice-eating areas e g in parts of China Japan, southern India and the East Indies. During the period 1928–32 10 000–20,000 deaths from beri-beri were recorded yearly in Japan In the Philippines, in the 1940 s, 24,000 deaths occurred in a single year (Table XV)

TABLE XV *Deaths from beri-beri in the Philippines*

Year	No of deaths per 100,000 inhabitants
1939	116·13
1940	111 87
1946	148 21
1947*	131·92

* In 1947 the number of deaths from beri-beri was 24,296 two-thirds of the deaths were among infants beri-beri ranked second to pulmonary tuberculosis as a cause of death.

DO WE HAVE ENOUGH VITAMIN B_1

Beri-beri is practically unknown in Europe.[1] That does not necessarily prove that some people may not be *partially* short of vitamin B_1 and would be in better health if they had a little more. In fact, tests in the United States and in Canada have proved that infants and children have sometimes put on weight better with extra vitamin B_1. We shall discuss this matter of 'partial deficiencies' later, both at the end of this chapter and again in the last chapter of this book.

So much for beri-beri in man.

BERI-BERI IN ANIMALS

The accidental discovery of beri-beri (or 'polyneuritis') in hens by Eijkman in the Dutch East Indies in 1897 has already been mentioned. It has since been found that pigeons and other birds also develop similar symptoms when fed on a diet of polished rice. Fig. 14 shows how the pigeon looks in a state of collapse and with its head retracted (the condition is called opisthotonus) and we see also the astonishing effect of giving vitamin B_1: recovery being complete within a few hours. So far as is known *all* species of animals seem to need vitamin B_1, not only man, pigeons, and hens, although as we shall learn (p. 51) some of them are able to make their own, or rather get it made for them by beneficent microbes present in their stomach or bowels.

Pigeons and rats are used mostly for studies on the vitamin. When fed on a diet containing too little vitamin B_1 the rat soon begins to lose weight and later may develop convulsions. Give him some vitamin B_1 and although he is at the point of death he makes a prompt and complete recovery (Fig. 15).

REFECTION

This technical-sounding heading refers to a peculiarly interesting phenomenon. Sometimes a rat which ought to be showing signs of lack of vitamin B_1 (having none in his diet) remains mysteriously normal. This disconcerting

[1] Apart from occasional cases of 'conditioned' deficiency (p. 72).



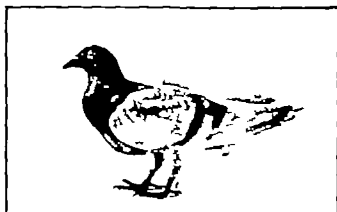


Fig 14. Before and after The spectacular effect of a fragment of vitamin B_1 given to a pigeon with beri-beri.



(1) (2)

Fig 15 Rats fed on (1) white flour (contains no vitamin B_1) (2) wholemeal (contains the vitamin) First rat has paralysis and convulsions, curable by vitamin B_1 second is normal.

happening is found to be due to the fact that he is getting large amounts of raw uncooked starch in his diet. This starch is incompletely digested, and in consequence ferments in the intestine. The result is that certain microbes there are able to flourish, and one of their special occupations is to make various vitamins of the 'B' group. This proves very convenient for the rat, for he can make use of this gift of the vitamin which the microbes have manufactured for him, and so he is able to remain in perfect health.

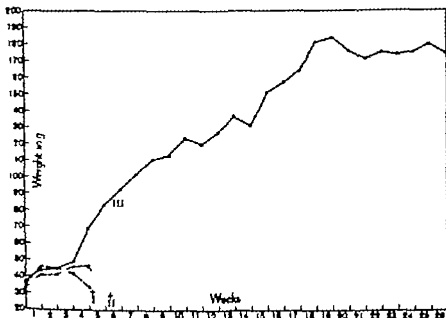


Fig. 16. Growth chart of a 'refected' rat.

Two rats, I and II, on a diet containing no vitamin B_1 lose weight and die because of the deficiency. But a third, III, becomes spontaneously 'refected' and so survives (Fridericia et al.)

Cows and other ruminants, however, who have to consume large amounts of coarse fibrous vegetable food, are in a state of permanent refection. The fermenting mass of food residue both in their rumen and in the bowel, makes an ideal breeding-ground for microbes. These furnish the host with a continuous supply of ready-made vitamin B_1 and of other 'B' vitamins too. Hence it is of little consequence to the health of the cow, or to the vitamin-B

value of her milk, whether the B vitamins are present in the food or not Similar conditions apply to rabbits, and certain other species.

So far as is known man cannot get refected (as it is called) in this way If he could, it would be a simple way of preventing beri beri! Nevertheless, it seems that a variable if small amount of vitamin B_1 may normally be produced by micro-organisms in the intestine of the human subject.

HOW VITAMIN B_1 WAS ISOLATED

The most characteristic trait in all scientists I think, is their inquisitiveness—or shall we say their curiosity? Naturally therefore the chemist is not satisfied until each vitamin has been isolated and its formula worked out. Finally the vitamin must be made artificially in the laboratory from its component parts, synthesized. Only when this has been done are we in a position to understand properly all sorts of questions about the vitamin and how it works.

For vitamin C all this including the actual synthesis, was already accomplished in 1933 For vitamin B_1 the final stage was reached in 1936 and for vitamins E K and A in 1938 1939 and 1946 respectively Vitamin D has not yet been made synthetically (See Chapter II Table IX.)

The isolation of the pure crystalline vitamin B_1 was an extremely difficult task, and many years were to pass before it was to be finally achieved. Numerous contributors had to lend a hand and help the work on a little and then drop out and leave it for others to pursue still further This gradual progress is seen from Table XVI

TABLE XVI *Progress in concentrating the anti-beri-beri vitamin*

Year	Investigator	Dose needed (milligrammes)
1912	Suzuki	5
1912	Eadie	3–6
1913	Funk	4–8
1918	Abderhalden	5
1924	Seidell	2
1926	Jansen & Donath	0·012

The method of isolating a vitamin like that of any other natural substance present in a foodstuff or in animal or vegetable matter involves treating the latter in turn with numerous precipitating agents, solvents and so on and thus gradually removing everything else step by step until finally only the vitamin remains. As everything else may amount to several thousand parts and the vitamin to only one part the complexities of the chemist s work may be imagined After each operation an animal test had to be carried out to see what had happened to the vitamin whether for example it had got carried down on the precipitate or remained in the solution Then another precipitation had to follow to separate it still further from other things. As many animals had to be used for each single test and it all took time the whole thing was extremely laborious and all except the most patient and skilful of investigators were likely to lose heart

Finally in 19 6–7 Jansen & Donath two Dutch workers, separated some crystals which appeared indeed to be the vitamin itself in a pure condition By a happy chance they were working in that same laboratory in which Eijkman had done his original pioneer work a quarter of a century and more earlier



Fig 17 A crystalline vitamin preparation.
Vitamin B_1 chloride-hydrochloride (Jansen & Donath).

What made it certain that Jansen & Donath had in fact got the pure vitamin itself free from any contaminating substance, was that other investigators since working in other parts of the world, and also trying to i olate the vitamin always ended up with a material which was identical with that of Jansen & Donath as shown by elaborate tests carried out on it, using various physical, chemical and physiological criteria. Among these other investigators we may mention Windaus in Germany Otake in Japan Van Veen in Java Peters in Oxford

A picture of Jansen & Donath s crystals is shown in Fig 17 It has the

formula $C_{12}H_{17}ON_4SCl$ HCl and Jansen suggested for the pure substance the chemical name ancurin (short for anti-neur-itic vitamin) (An alternative name, thiamine was later introduced in the United States, and won international acceptance in 1951)

From Table XVII it will be seen how complicated and long was the process needed to accomplish the separation

SOME PROPERTIES OF VITAMIN B_1

It has been found that when foods containing vitamin B_1 are heated, above the boiling-point of water their activity is soon lost—the vitamin is destroyed —the more so if the solution is at all on the alkaline side acid helps to

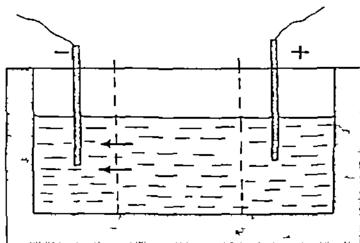
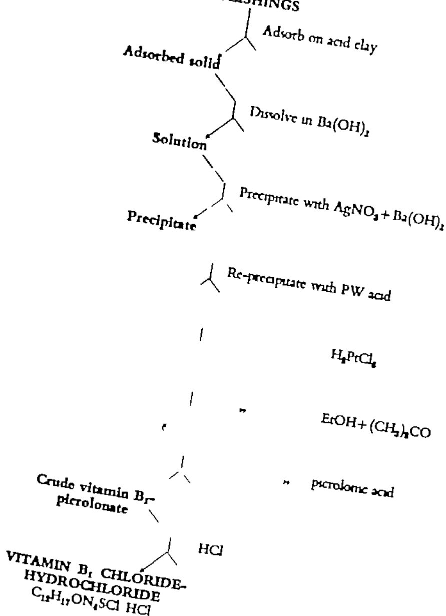


Fig 18 Proof that vitamin B_1 is a base. Electrodialysis apparatus. Vitamin-B_1 activity travels to negative pole Hence vitamin B_1 is positively charged (Birch & Guha, 1931)

protect it. (Also a perfectly dry food can stand the heat better than one that is moist.) *But vitamin B_1 is on the whole somewhat less easily destroyed* by heat as in cooking than is vitamin C The latter is in most ways the most unstable of all the vitamins.

Chemically speaking vitamin B_1 is peculiar in containing sulphur (notice the formula, p 56) this unexpected discovery was made by Professor

TABLE XVII *Isolation of vitamin B_1*
(Jansen & Donath, 1927)



Windaus and his colleagues at Göttingen. It is not an acid on the contrary it is a base This fact was first conclusively proved by two colleagues of mine, Messrs Birch & Guha, who showed how it behaved under an electric current (Fig 18)

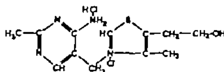
As we know vitamin B_1 can be dissolved easily in water and in alcohol and in most acid solutions It does not dissolve in fats When fuller s earth or charcoal, or certain other powders are thrown into a solution containing the vitamin the vitamin is removed from the solution and adheres to the surface of the powder or as we say it is *adsorbed* This fact is made use of in the processes for isolating the vitamin

THE STRUCTURE OF THE MOLECULE

The final step in the chemical study of vitamin B_1 was to determine how the atoms are disposed in the molecule of the vitamin

A comprehensive and brilliantly successful attack on the problem of the structural formula of the vitamin was undertaken by Professor R. R. Williams and his fellow-workers at Columbia University and simultaneously by Grewe in Germany as a result of which accounting for all the known facts about its chemical behaviour it was possible in 1935–6 to propound the formula shown in Table XVIII This tells the chemist that the molecule of

TABLE XVIII *The molecular structure of vitamin B_1 chloride-hydrochloride* (Williams s formula, 1936)



the vitamin is built up of two ring systems namely a pyrimidine and a thiazole system It is of interest to find that it is the former which is the more weakly basic group and the latter the more strongly basic group with the pseudo-acid properties —features which Dr Birch and I had independently deduced in 1934 from a study of the *titration curve* of the vitamin. Finally late in 1936 came the news of the final achievement in the chemist s attack

on vitamin B_1—its artificial synthesis in the laboratory this was accomplished, independently and almost simultaneously both in America and in two separate institutes in Europe

WHICH FOODS CONTAIN VITAMIN B_1?

Many natural foods have been found to contain a moderate amount of vitamin B_1 But none has it in *very high* concentration This fact rather distinguishes it from certain other vitamins which on the contrary are to be found much more concentrated in certain selected foods For example a tablespoonful of cod liver oil (or even less of halibut liver oil) taken every day would give ample vitamin D even if all the other articles in the diet contained none at all and would therefore prevent rickets and an orange a day by itself contains enough vitamin C to prevent scurvy

Wheat embryo and the germ of other cereals as well as bran are all good sources of vitamin B_1 White bread has practically none but it is present in wholemeal flour or bread (containing 100 per cent of the original wheat grain) or in the war time National Wheatmeal (containing about 85 per cent of the grain) as well as in certain proprietary flours enriched with added germ [1] The most potent of all natural products for vitamin B_1

TABLE XIX *Amount of vitamin B_1 in different foods*

Name of food	Approximate vitamin-B_1 content (microgrammes per 100 grammes of food)
Dried brewer's yeast	6000–3000
Wheat barley or rice-germ	3000
Oatmeal	900
Wholemeal wheat, peas haricot beans, egg yolk	300

* 1 microgramme = 1 μg = one-millionth of a gramme

[1] Pre-war white bread had an extraction rate of about 72 to 75 per cent. See p 317

is yeast (including dried Food Yeasts) and other good sources include peas, beans and wholemeal cereals egg yolk, liver kidney or heart. Numerical values—in approximate round numbers—are given in Table XIX, but of course it must be realized that different samples of a food may vary somewhat in their activity

The daily requirement The amount of vitamin B_1 needed by a human being to prevent or cure beri-beri and maintain normal health, is of the order of 1000 microgrammes per day (that is, one milligramme or about one thirty-thousandth part of an ounce avoirdupois) [1]

MEASURING THE VITAMIN VALUE OF FOODS

It is obviously important to be able to know the amount of vitamin B_1 and of the other vitamins in different foodstuffs During the War of 1939–45 many dozens of preparations were assayed at the Nutritional Laboratory Cambridge, for the official guidance of Government Departments In London, a laboratory maintained by the Pharmaceutical Society used to devote its full time to carrying out routine work of this kind testing foods for various manufacturing firms and public authorities.

Until about 1937 no chemical method was available for analysing food stuffs for vitamin B_1 so tests on animals had to be used. Such animal tests saved hundreds of lives in the past in the East by showing which foods were suitable for use as remedies and preventives of beri-beri

To measure the amount of vitamin B_1 in a foodstuff by biological test, rats or pigeons are taken already suffering from the symptoms of shortage of vitamin B_1 Graded doses of the material under test are then administered to these animals by groups Perhaps a group of four rats are given 1 gramme each another four rats a dose of 2 grammes each another 4 grammes each, and so on One can then note the amount which is needed to bring about a cure lasting a particular time (or which results in a specified rate of growth in the animal) (Fig 19) This dose has then to be compared with the amount of a *standard material*, given at the same time to *another* group of rats, needed

[1] For official standards of vitamin requirements, see pp 315 352.

to produce the same effect In this way one can say that so many grammes of the food are equivalent to so many units of standard.

The League of Nations standard The standard referred to was chosen by an international conference convened by the Health Organization of the League of Nations At first the standard was a concentrated preparation of vitamin B_1 Later it was possible to substitute an extremely pure preparation of synthetic vitamin B_1 itself One *international unit* of vitamin B_1 is fixed by definition

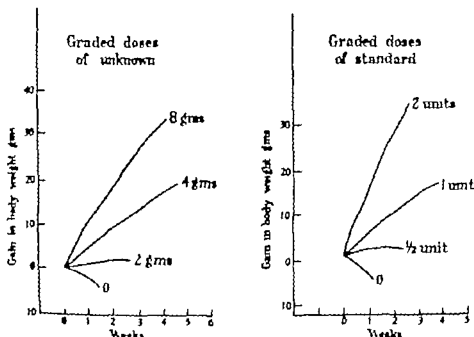


Fig 19 Assaying a foodstuff for vitamin B_1 potency by the growth-rate method.

as the potency of 3 microgrammes of this international-standard preparation (One microgramme is a millionth part of a gramme One gramme is approximately the 28th part of an ounce avoirdupois)

The advantage of having the international standard is that now foodstuffs examined in different parts of the world may all be measured against a common yard-stick It would clearly be less satisfactory to give the result as the amount of the food necessary to protect a rat or a pigeon for so long—because obviously rats and pigeons may vary from one to another and also

calculating the result by drawing dose-response curves and comparing their steepness is shown in Fig 22

Scientists in various parts of the world have made use of this heart-rate test for vitamin B_1 For example, it has been used for checking the activity of synthetic preparations of the vitamin for testing pharmaceutical concentrates and natural products for examining the distribution of the vitamin in

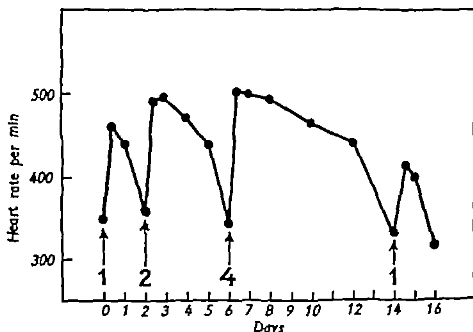


Fig 21 Temporary cures of the low heart-rate in a vitamin-B_1-deficient rat. The bigger the dose of vitamin B_1 given (marked at arrows) the longer the duration of the resulting cure.

the tissues of the body or its secretion in specimens of *human milk*, and its excretion in the urine for comparing the vitamin contents of various types of breads and flours for finding the effect of different manurial treatments on the vitamin value of crops for measuring the rate of production of the vitamin by bacteria and, lastly for checking the reliability of the newer chemical methods of vitamin analysis that have since come into use

MEASURING VITAMIN B_1 BY CHEMICAL TESTS

In the early days of vitamin research each vitamin was known only by the effects of its deficiency The existence of vitamins was still only a theory and not yet a proved fact One, the anti beri beri vitamin was postulated to be concerned in the prevention of beri-beri another in the prevention of scurvy

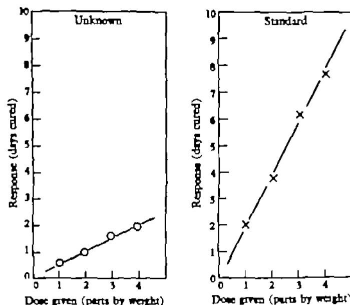


Fig 22 Dose-response curves, as used for computing the vitamin-B_1 value of a given foodstuff o vitamin preparation.

In the example here shown, the Unknown was a commercial vitamin preparation. Its curve of response, on left, was found to be one-quarter as steep as that of the International Standard, on right. Hence it could be concluded that, weight for weight, the preparation in question had one-quarter of the activity of a pure specimen of vitamin B_1

and so on Hence the earliest method of examining a food for the presence or absence of a given vitamin was always by a test on animals Later little by little, as more became known about the chemical nature of vitamins it has proved possible to develop purely chemical tests The best known and most useful chemical test for vitamin B_1 is called the thiochrome test, because it depends on the conversion of vitamin B_1 by an appropriate process of oxidation

(treatment with an alkaline solution of *potassium ferricyanide*) to a related substance called thiochrome (Table XXI) The amount of thiochrome formed (after suitable separation and purification) is measured by its fluor escence—that is to say by seeing how much luminosity it excites when exposed to ultra-violet rays This thiochrome test was introduced in 1936 by the Dutch chemist Jansen who as we have learned was also the first to isolate







Fig 23 Fig 24

Fig 23 Electrocardiogram for measuring the heart-rate of a rat used for determining the vitamin-B_1 potency of a foodstuff.

Fig 24. Tracings of heart-beat of normal and of vitamin-B_1 deficient rats (above and below respectively) The heart returns to normal overnight after a dose of vitamin B_1 has been given.

pure vitamin B_1 The test has gradually been refined and improved in different respects by various people in other parts of the world in turn. One set of modifications introduced by Dr Wang and myself has been officially adopted for the testing of National wheatmeal flour (to ascertain that it conforms to the government regulations) and by the *British Pharmacopoeia* for checking the purity of specimens of crystalline vitamin B_1 To be sure

that the chemical method gave reliable results we compared our figures with those we got in three separate kinds of animal tests. The agreement was good (Table XXII) which proves that the chemical test is satisfactory A test by this chemical method can be completed in minutes or hours instead of as many weeks or months needed for tests with animals This has been a great

TABLE XXI *Chemical structure of thiochrome a substance closely related to vitamin B_1 (compare Table XVIII) and used for its detection and measurement*

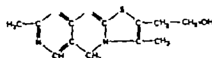


TABLE XXII *Agreement between chemical and biological assays*

Some typical materials tested (arranged in descending order of activity)	Vitamin-B_1 content (as no of international units per gramme)			
	Chemical test (thiochrome method)	Biological tests		
		Bradycardia method	Cure of convulsions in rats	Growth rate of rats
High-potency yeast Specimen 1	200	191	—	—
" " 2	57	66	—	—
Dried yeast	—	19	19	20
Wheat germ Specimen 1	9	9	—	—
" " 2	—	7	7	7
Yeast extract	7	7–8	6	7
Dried egg yolk	2·4	2·7	—	—
Whole wheatmeal Specimen 1	1·7	1·7	—	—
" 2	1·3	1·2	—	—
" " 3	1·2	1·1	—	—
Skimmed milk powder	0·9	0·9	—	—
Pearl barley	0·4	0·4	—	—
Wheatmeal biscuit	0·3	0·3	0·3	0·4
Potato Majestic	0·3	0·4	—	—
Cauliflower	0·3	0·3	—	—
Herring, raw	0·03	0·04	—	—

boon and has permitted knowledge to develop much more rapidly than would have been possible with the more cumbersome animal tests. In the case of any doubt, however the test of potency on animals remains the final criterion

At the present time several other chemical tests are being gradually introduced and improved.

TESTS WITH MICROBES

A still later development has been to estimate vitamins by their stimulating effect not on large animals such as rats or pigeons but on micro-organisms. This has the advantage of being both more economical and more rapid, because, first microbes consume less of the material under test and secondly they grow faster One such method is to compare the rate of growth of the microbes in question with and without the addition of different amounts of the vitamin or food containing it. An alternative plan is to measure the activity of the microbes indirectly either by ascertaining the rate at which they are using up certain chemicals which serve them as their food, or else by measuring the amount of certain other chemical by-products which they throw off.

The best of these micro-biological methods for vitamin B_1 is one perfected by Schultz in America. In this the micro-organism used is yeast. Measurements are made of the rate at which under controlled conditions, the added vitamin promotes fermentation by the yeast, which is indicated by the rate of evolution of the gas carbon dioxide. This is another promising method of test.

At any rate, the investigator on vitamins has now no shortage of reliable, alternative methods to choose from

HOW DOES VITAMIN B_1 WORK ?

In the history of research on each vitamin one of the last (because one of the most difficult) questions to be answered has always been How does it work? Now the body can be regarded as an intricate chemical factory where hundreds of different kinds of chemical reactions are continually in progress

interconnected and interrelated one following another in the most complex and yet orderly manner Over which chemical reaction then does vitamin B_1 preside? In other words precisely which chemical reaction in the body gets out-of-gear when vitamin B_1 is absent? In the earlier period of investigation on these problems numerous alternative theories were put forward none of them backed by much experimental evidence and therefore none of them very convincing Two clues however seemed specially significant One was the observation that excess of sugar or starch or other carbohydrate food, intensified the symptoms of beri-beri in pigeons whereas fat was vitamin-sparing Perhaps then the vitamin was concerned in some special way with carbohydrate? Secondly when an animal developed beri-beri there seemed evidence of some impairment in the oxidative processes by which the animal body gains its energy and warmth

We have since learned that the correct explanation is indeed a combination of these two earlier theories Vitamin B_1 is concerned, first in one of these processes of combustion (*oxidation*) occurring in the body and secondly and more specifically in the chemical transformations (*metabolism*) of carbohydrates—i.e starches, sugars, and the like To be more precise it was later proved that the vitamin is needed for the disposal and breakdown of a particular substance, *pyruvic acid* which is formed at one stage in the breakdown of carbohydrates Thus when vitamin B_1 is absent, this step in the orderly sequence of chemical changes in the body is held up and the consequence is that other chemical errors also follow in its train. In particular two products accumulate in the body in excessive amounts when the vitamin is absent namely the pyruvic acid already mentioned and also another closely related substance *lactic acid* We may pause for a moment to recall how all this knowledge has gradually been pieced together

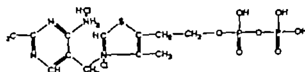
UNRAVELLING THE CHEMICAL FUNCTION OF VITAMIN B_1

The first link in the chain of knowledge was forged in 1922–5 when investigators from Montevideo and from Berlin named Collazo and Bickel noticed that excessive quantities of one of the products just mentioned, **lactic acid**

could be detected in the blood and urine of animals with beri-beri. In Japan, Hayasaka, found that when dogs or patients with beri-beri were made to do physical exercise, the lactic acid in their blood stayed at a high level for a long time instead of rising only temporarily and then rapidly falling again—as it does with a normal subject Then at Oxford Professor R A Peters pointed out that the brains of pigeons developing symptoms of beri-beri accumulated abnormally large amounts of lactic acid. Moreover when he conducted experiments on a minced specimen of such a pigeon brain in a test-tube, he found that it had lost to some extent its vital function of taking up oxygen, especially when lactic acid was added to it This could be restored by adding some vitamin B_1 to the test-tube, so as to replace the missing factor Thus the vitamin was clearly concerned in maintaining the normal oxidative properties of the cells of the body in the presence of lactic acid.

Later attention came to be turned from lactic acid towards **pyruvic acid.** It was the work of a noted German-Jewish scientist and Nobel laureate, Professor Otto Meyerhof that had shown the importance of pyruvic acid alongside with lactic acid in the biochemistry of carbohydrate metabolism. Tests carried out soon showed that excessive amounts of pyruvic acid, and not only of lactic acid, could be detected in the blood or urine of animals with beri-beri. The same was true of human patients as was recorded by an Englishman B S Platt, working with Miss G D Lu in China. Equally vitamin B_1 was necessary in Peters s test-tube experiments if a proper uptake of oxygen was to be conferred on pyruvic acid when it was added to minced brain tissues from a pigeon with beri-beri. Thus vitamin B_1 in some way helped in the chemical transformations of pyruvic acid.

TABLE XXIII. *Chemical structure of co-carboxylase the form in which vitamin B_1 functions in the body It is the di-phosphate ester of vitamin B_1 (Compare the formula of vitamin B_1 itself in Table XVIII)*



The crucial clue to the whole problem was provided in 1937 by Lohman and Schuster in Germany What they proved was that it is vitamin B_1 in the form of its di-phosphate ester (Table XXIII) which fulfils the function of a certain co-enzyme co-carboxylase What this means can be expressed in relatively non technical terms as follows

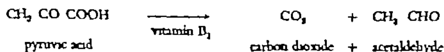
VITAMINS AS FERMENTS

Enzymes (more popularly known as ferments) or biochemical catalysts are a class of substances which when present in the merest traces, can by their influence initiate chemical reactions and cause them to continue. Figuratively they are the triggers of chemical reactions or the whip which impels the chemical motion In a more literal sense enzymes can be compared with the trace of leaven which raises the whole lump—for indeed it is these ferments or enzymes in the yeast which cause the fermentation and hence the rising of the dough. Now among the ferments in yeast there happens to be one called by the biochemists, *carboxylase* and this it is which is our immediate concern. Its function is to set in motion a particular chemical action leading to the liberation of carbon dioxide from the substance *pyruvic acid* to which we have already been introduced But in order for this *enzyme* system—carboxylase—to function it needs to have a partner or *co-enzyme—co-carboxylase* And it is this co-enzyme that as we have just learned, has been identified as the di-phosphate ester of vitamin B_1

This is the crux of the whole matter

VITAMIN ACTION IN YEAST AND MAN

In a word then in yeast vitamin B_1 di-phosphate alias co-carboxylase serves as the co-enzyme concerned with the liberation of CO_2 from pyruvic acid. For the chemist we can write down a quite simple equation for this



$$\underset{\text{pyruvic acid}}{CH_3\,CO\,COOH} \xrightarrow[\text{vitamin } B_1]{} \underset{\text{carbon dioxide}}{CO_2} + \underset{\text{acetaldehyde}}{CH_3\,CHO}$$

Now the chemistry of carbohydrate breakdown in the human body is known to differ in many respects from that in yeast Nevertheless in essentials the action of vitamin B_1 seems to be the same. Once again, putting it in terms intelligible to the professional chemist vitamin B_1 as co-carboxylase, appears to be able to catalyse, perhaps indirectly many chemical changes in the animal body involving for example such diverse reactions as *oxidation dismutation* and *condensation* yet probably all involve at some stage the key feature of DECARBOXYLATION (i e loss of CO_2) There thus seems to be an underlying unity of concept about the mode of action of vitamin B_1 whether for yeast or for man which is very satisfying

We may now return to some considerations concerning the practical importance of vitamin B_1 for human beings

PARTIAL DEFICIENCIES

Passing allusion to the possibility of partial deficiency of vitamin B_1 has already been made (p 49) What is meant by the phrase can perhaps best be explained by a chart illustrating an experiment on animals If a rat is given no vitamin B_1 it will soon begin to lose weight steeply and then after three or four weeks will die from beri-beri (Fig 25) If given instead a small but still insufficient dose say one-quarter of a unit of the vitamin daily it will continue to grow for a somewhat longer period but will still succumb to beri-beri, although a little later With somewhat more again say half a unit daily the rat will perhaps remain just free from beri-beri it will, however still not be in good physical condition as shown by the fact that it will fail to put on weight sufficiently Double the daily allowance again to one unit daily and the weight curve will now be fairly satisfactory Double it yet again and there will still be some further gain in physique (the growth curve is steeper) while with even a further doubling of the dose some improvement may just still be perceptible. In scientific jargon, the dose response curve is almost logarithmic (Fig 25) From the practical point of view the essential fact is that several times more vitamin is needed for optimal health than for mere protection from severe deficiency disease There is little doubt

hat similar considerations apply to man We may therefore use the following terms

Avitaminosis is the severe disease resulting when there is little or no vitamin in the diet.

Hypo-vitaminosis is the more vague state of poor health when there is some of the vitamin in the diet but not enough.

The *minimal dose* of the vitamin is the bare amount required just to prevent symptoms of open deficiency disease

The *adequate* dose is the dose needed for reasonable health.

The *optimal* dose is that which gives health so perfect that a further increase will give no further perceptible improvement.[1]

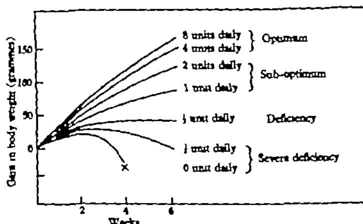


Fig. 25 The gap between the minimum and the optimum.

The minimum requirement is the amount of the vitamin needed to prevent the more obvious clinical signs of deficiency the optimum is the amount needed to promote maximum health.

See also Fig. 103 p 335 Chapter XIII.

ASSESSMENT OF NUTRITIONAL STATUS OF MAN IN VITAMIN B_1

It is sometimes useful to be able to know whether a schoolboy or a patient in a hospital or some native of a region where beri-beri is endemic, has or has not been having sufficient vitamin B_1 in his past diet—in other words

[1] It is assumed that there is at the same time an optimal supply of all other nutrients so that there is no question of a deficiency of some other substance being the limiting factor and so masking the effect of the extra vitamin by holding back the response

whether or not he is in a good nutritional state with regard to the vitamin. For this purpose I worked out (1936 1943) a test depending on an examination of specimens of his urine. In principle it is the same as that which I had previously devised for vitamin C (p 140) People whose diet has been relatively poor in the vitamin lose less of it in their urine and moreover when they are given test doses of the vitamin every day since their body needs it more urgently a day or two elapses before as much overflows in their urine. At first the only method we had for examining the urine was by finding its effect on the heart-rate of rats (the bradycardia test p 61) now we are able to use the much easier chemical test (p 63) Another procedure which I introduced for assessing the B_1-status depends on the fact, already explained, that the carbohydrate metabolism of the body becomes deranged when the vitamin-B_1 intake is inadequate. We give a loading test, namely a big dose of glucose or other appropriate substance, in order to put an extra strain on the carbohydrate metabolism. If this is followed by the appearance in urine or blood of unduly large amounts of pyruvic acid and related intermediates it means that the vitamin-B_1 supply is insufficient to fulfil its function of disposing of these substances. This test has been used especially in America by McCollum in observations on animals, and by Shils and by Bueding and by Williams and their collaborators for clinical purposes.

CONDITIONED DEFICIENCY

It occasionally happens that a patient develops symptoms of polyneuritis, or we might say beri-beri, even when there is enough vitamin B_1 in the usual staple dietary The explanation is that, for some reason or other he is not able to absorb the vitamin normally from his food, or else is not able to utilize it in his body or possibly he needs more than the usual amount This condition is called a conditioned deficiency (conditioned polyneuritis, conditioned beri-beri) because the deficiency is conditioned by or depends upon some other abnormality Three special kinds of conditioned deficiency of vitamin B_1 are now well recognized and are worth briefly noting here, namely *alcoholic neuritis neuritis of pregnancy* and neuritis associated with gastro-intestinal obstructions

Alcoholic neuritis a condition not uncommon in chronic inebriates was formerly thought to be due to the direct poisoning action of the alcohol on the nerve cells of the dipsomaniac However tests on urine proved that there was a genuine deficiency of the vitamin and administration (if necessary by injection) of large doses of the vitamin have resulted in spectacular cures The explanation here is partly that the alcoholic subject has so pickled his digestive system that it no longer functions adequately and the vitamin is not absorbed and partly that he obtains so large a proportion of his food-fuel from his alcohol that he takes insufficient food of the normal kind, containing the vitamin

In pregnancy the needs for vitamin B_1 as indeed for all nutrients are increased. In addition morning sickness may cause some loss of food There may also sometimes be mechanical difficulties interfering with the easy assimilation of the diet In extreme cases *polyneuritis of pregnancy* may be caused in this way and it can be successfully cured or prevented with synthetic vitamin B_1

Finally with disorders causing *chronic obstruction of the stomach or intestines* such as the surgeon may have to deal with there is again the possibility of faulty absorption and then vitamin B_1 in extra large doses or by injection, may be needed to cure the resulting conditioned deficiency

CHAPTER IV

PELLAGRA AND NICOTINAMIDE

Most people in Britain are rather mystified when one alludes to pellagra. They do not know that in the U S A. thousands of persons have died from it year after year

OVER 7000 DEATHS ANNUALLY FROM A PREVENTABLE DISEASE

In 1915 11 000 deaths from pellagra were recorded in the official returns from the southern states of the U S A the actual number of deaths was said to be much higher for many deaths in fact due to the disease do not find their way into the official statistics. Some figures for the third decade of this century are shown in Table XXIV

TABLE XXIV *Deaths from pellagra as reported in U S Census Department*

Year	No of deaths
1924	2006
1925	3526
1926	4315
1927	5148
1928	7502
1929	7367
1930	7086

Why did over 7000 people die in 1930 from a disease that was preventable? The explanation is economic, not scientific. The deaths were largely among negroes and poor whites almost entirely in southern states, and were the result of economic depression. It is the food which is to blame. The victims of pellagra (*pellagrins* as they are called) subsist on a diet known as the three M s —Maize-meal, Molasses and Meat (salt pork) Such a regimen contains an insufficiency of the anti-pellagra vitamin. It was a sad commentary on our

civilization that while these people were dying from a dietary deficiency at the same time in other parts of the continent food was being burned or thrown into the sea because of over-production

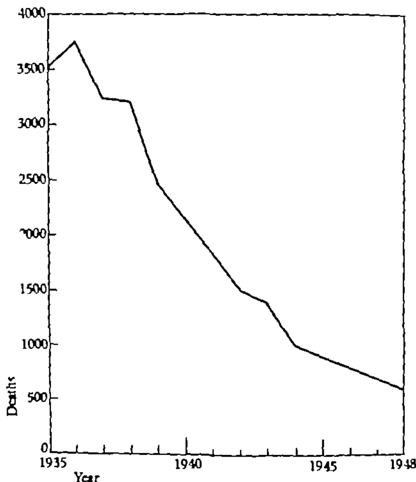


Fig 25A. Number of deaths from pellagra in the U S.A., 1935–1948
(Deaths from alcoholic pellagra—see p 77—are excluded from the totals shown above.)

However that may be we may quote a few further statistics just to drive the matter home and show that in America pellagra has not been a thing to overlook

In 1917 over 170 000 cases of pellagra occurred in the southern states in 19-7 over 120,000 cases

In 1916, pellagra ranked second as a cause of death in South Carolina or third or fourth in Mississippi and Alabama The Metropolitan Life Insurance Company of America recorded more deaths from pellagra than from general tuberculosis or malaria during 1911–16

It is reassuring to learn that, in more recent years there has been a fall in the pellagra death-rate curve for the U S A (Fig 25A)

PELLAGRA ELSEWHERE

Pellagra does occur in other parts of the world too as in Italy Roumania Africa and in fact wherever peasant populations are forced to rely too exclusively on maize as their staple food. So common is it in Egypt as to justify the observation that it is high in the list of the modern plagues of Egypt Fortunately it is generally less severe outside America, possibly because the maize is less thoroughly milled and therefore retains some of its original vitamin, or more likely because the diet is not quite so one sided.

WHAT PELLAGRA LOOKS LIKE

As the diet (in America) is the three M s so the symptoms have been called the three D s —dermatitis, diarrhoea and dementia The dermatitis, or inflammation of the skin is the most characteristic feature and once seen is not easily forgotten It appears in strangely symmetrical areas equally on both sides of the body (Fig 26) The skin becomes bronzed and dusky resembling sunburn (pp 78–9) and afterwards greatly thickened. Areas of the body exposed to sunlight—face, wrists neck—or to friction—under the legs—are especially affected. Fancifully the appearance is described as the pellagra glove (on the hand) the pellagra shoe (on the foot) the pellagra collar (round the neck) and the butterfly wing (from the shape of the inflamed area on the face) The tongue also has a typical appearance, swollen glossy and beefy The unfortunate victim generally has mental troubles progressing to full insanity in the last stages of his affliction unless he is cured in time.

NON ENDEMIC PELLAGRA

Sporadic cases of pellagra are not uncommon in mental asylums The reason is either that the diet is unsatisfactory or more often that the patient has fads and fancies about his food and refuses to eat some of the more nourishing articles provided. Alternatively people already with mild pellagra may find their way into asylums because they have developed mental symptoms. There is thus a kind of vicious circle pellagra may cause mental symptoms and mental disease may cause pellagra A case seen by the writer in a mental institution is shown in Fig 26 (4)

In many backward countries pellagra is common, too in prisons because the food given is so disgracefully inadequate.

A conditioned deficiency of the anti pellagra vitamin may also cause occasional cases to be seen in hospital. Like conditioned deficiency of vitamin B_1 (already mentioned on p 72) it is due to faulty absorption or assimilation for example in chronic alcoholism or with some obstructive trouble in stomach or bowels

But such kinds of pellagra are exceptional and the truly serious problem is in those regions where it is endemic, and where cases occur by the thousand—because the people are poor and their food is in consequence unsatisfactory in quality or quantity or both.

TRACKING DOWN THE CAUSE OF PELLAGRA

The scientist who did most to work out the cause of pellagra was Joseph Goldberger a Jewish-American physician in the Public Health Office of the United States.

The first thing to prove was that pellagra was not due to bad hygiene or infection, as was popularly supposed, but to bad diet This Goldberger did in two ways. In the first place he looked into the diet sheets and he was able to show that those who became ill had been living on a poorer diet than those in the same district who remained well. Those who escaped the pellagra ate more *fresh meat eggs* or *milk* than did those who succumbed.



(1) A negress from S Carolina with the typical pellagra glove symptom.



(2) Pellagra in a child. Note the butterfly wing on the face, and the rough hands.



(3) An Egyptian case Notice the inflammation of the skin on face hands, neck and upper chest (the pellagra collar)



(4) Pellagra in an asylum.

A case of pellagra seen in a mental hospital in England.

Fig 6. Pellagra in the U S.A. and elsewhere.

Many thousands of people suffer from this preventable and curable disease and about 7000 of them died from it every year between 1927 and 1931—starvation in the midst of plenty

The next link in the argument was more striking still. Goldberger was able to show that he could invariably cure pellagra at will, or prevent it, or even produce it experimentally in human beings by making suitable changes in the diet. This is how it was done

AN EXPERIMENT ON HUMAN BEINGS

Two orphan asylums and a lunatic asylum where pellagra had been common were chosen for the first test. Goldberger improved the diet adding more meat, vegetables, fruit and eggs The experiment was completely successful. Not one case of pellagra occurred on the improved diets, while those who had previously suffered from pellagra on the old diet (upwards of 200 in all) were rapidly restored to normal health.

PRODUCING PELLAGRA IN HUMAN VOLUNTEERS

The next experiment was a bold one. A group of convicts in an American gaol were offered pardons if they would volunteer to live on a diet modelled on that of the cotton-mill workers in the Mississippi area, where pellagra was so prevalent. Eleven prisoners underwent the test, and by the end of five months five of them had developed the skin lesions considered typical of incipient pellagra. This was regarded as pretty conclusive evidence to prove that pellagra was not an infection.

Goldberger and fifteen of his associates next tried in a number of ways to transmit pellagra to themselves by inoculations with blood nasopharyngeal secretions faeces urine and desquamating epithelium The results of this heroic experiment were entirely negative Not a single one showed any evidence of the disease. This proved that pellagra was not catching

Goldberger could now be sure that pellagra was caused by something missing from the diet. He could not yet be sure what this something was. Only after further experiments was the existence of an anti-pellagra vitamin to be recognized. (For some time indeed it was generally supposed that pellagra was due to the inferior quality of the protein of the maize and there is after all something in this view as we shall learn later p 96)

Once again, animal tests were called in to help

THE ANTI-PELLAGRA VITAMIN

In 1920 an American physiologist Voegtlin by name had tried administering to his pellagra patients certain extracts rich in vitamin B —then supposed to be a single substance. To his satisfaction he found that the patients got better The only puzzling thing was that whereas these extracts were beneficial, as soon as he tried feeding a more purified preparation of anti-beri-beri vitamin itself the result was negative This seemed to show that this something which cured the pellagra accompanied the anti beri-beri vitamin in certain extracts yet could not be identical with it

In 1927 Goldberger made a similar observation that most foods which were rich in vitamin B would cure pellagra But he went one step further and tried to find out how the pellagra preventing vitamin differed from the anti-beri-beri factor To do this he made tests on dogs, and found that he could produce in them a condition which he named black-tongue and which resembled pellagra in man He was then able to show that the substance which cured this experimental pellagra in his dogs differed from that which cured beri-beri in three respects at least (1) it was not present in extracts prepared from maize-meal, which nevertheless were rich in the anti-beri-beri factor (2) it was not destroyed so easily by heating as was the anti-beri-beri factor and (3) it could not be extracted from foods so easily by alcohol Goldberger called it the P P or pellagra-preventive factor to distinguish it from the anti beri-beri, or A N (anti-neuritic) factor

RAT PELLAGRA IS NOT TRUE PELLAGRA

Unfortunately at this stage a little confusion entered into the history of the identification of the anti-pellagra vitamin and it was some years before the puzzle was fully cleared up It came about in this way As well as the experimental disease black-tongue in dogs which Goldberger had correctly identified as being the analogue of the pellagra of human beings, there was also another apparently similar disease which he succeeded in producing experimentally in rats This he called rat pellagra since he thought it likewise to be the equivalent of human pellagra He seemed justified in this

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In 19 7 Goldberger made a similar observation that most foods which were rich in vitamin B would cure pellagra But he went one step further and tried to find out how the pellagra-preventing vitamin differed from the anti-beri-beri factor To do this he made tests on dogs, and found that he could produce in them a condition which he named black-tongue and which resembled pellagra in man He was then able to show that the substance which cured this experimental pellagra in his dogs differed from that which cured beri beri in three respects at least (1) it was not present in extracts prepared from maize-meal which nevertheless were rich in the anti-beri-beri factor (2) it was not destroyed so easily by heating as was the anti-beri-beri factor and (3) it could not be extracted from foods so easily by alcohol Goldberger called it the P P or pellagra-preventive factor to distinguish it from the anti-beri-beri or A-N (anti-neuritic) factor

RAT PELLAGRA IS NOT TRUE PELLAGRA

Unfortunately at this stage a little confusion entered into the history of the identification of the anti-pellagra vitamin and it was some years before the puzzle was fully cleared up It came about in this way As well as the experimental disease black-tongue in dogs, which Goldberger had correctly identified as being the analogue of the pellagra of human beings, there was also another apparently similar disease which he succeeded in producing experimentally in rats This he called rat pellagra since he thought it likewise to be the equivalent of human pellagra. He seemed justified in this

The next link in the argument was more striking still. Goldberger was able to show that he could invariably cure pellagra at will, or prevent it, or even produce it experimentally in human beings by making suitable changes in the diet This is how it was done

AN EXPERIMENT ON HUMAN BEINGS

Two orphan asylums and a lunatic asylum where pellagra had been common were chosen for the first test Goldberger improved the diet, adding more meat, vegetables fruit and eggs The experiment was completely successful. Not one case of pellagra occurred on the improved diets, while those who had previously suffered from pellagra on the old diet (upwards of 200 in all) were rapidly *restored to normal health.*

PRODUCING PELLAGRA IN HUMAN VOLUNTEERS

The next experiment was a bold one. A group of convicts in an American gaol were offered pardons if they would volunteer to live on a diet modelled on that of the cotton-mill workers in the Mississippi area, where pellagra was so prevalent. Eleven prisoners underwent the test, and by the end of five months five of them had developed the skin lesions considered typical of incipient pellagra. This was regarded as pretty conclusive evidence to prove that pellagra was not an infection.

Goldberger and fifteen of his associates next tried in a number of ways to *transmit pellagra to themselves by inoculations* with blood, nasopharyngeal secretions faeces urine and desquamating epithelium The results of this heroic experiment were entirely negative. Not a single one showed any evidence of the disease This proved that pellagra was not catching

Goldberger could now be sure that pellagra was caused by something missing from the diet He could not yet be sure what this something was. Only after further experiments was the existence of an anti-pellagra vitamin to be recognized (For some time indeed it was generally supposed that pellagra was due to the inferior quality of the protein of the maize and there is after all something in this view as we shall learn later p 96)

Once again animal tests were called in to help

THE ANTI PELLAGRA VITAMIN

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conclusion for the signs of deficiency (a severe skin eruption on the rats hands feet and ears as well as a failure in growth) looked not dissimilar More over the factor that prevented or cured this rat pellagra was found in much the same kinds of foodstuffs Also like the anti-pellagra vitamin it was not destroyed readily by heat, and in this respect was markedly differentiated from the anti-beri-beri vitamin. Unhappily however Goldberger was wrong about the rat pellagra and it was some eight or nine years before the error was realized. We now know that the pellagra-like disease in rats—including their failure to grow and certain other abnormalities—was due to a deficiency of some other factor (or rather several other factors) generally found in food-stuffs alongside the true anti-pellagra vitamin but in reality quite distinct from it. Investigators were in consequence following the wrong trail for a few years afterwards in using tests on rats to try and guide them in their efforts to isolate the P-P factor Rat pellagra had in fact been wrongly named

CALLED VITAMIN B_2 BUT REALLY A COMPLEX

Not suspecting this complexity a committee in London proposed a new system of nomenclature, as a supposed improvement on Goldberger s own suggestion of A-N for the anti-neuritic and P-P for the pellagra-

TABLE XXV *Vitamins B_1 and B_2* compared* 1927

	Vitamin B_1	Vitamin B_2*
Wheat-embryo extract	A rich source	A poor source
Liver extract	A poor source	A rich source
Effect of heating in autoclave	Activity lost	Activity remains
Solubility in strong alcohol solution	More soluble	Less soluble
Effect of deficiency in rats	Loss of weight, polyneuritis convulsions	Loss of weight and skin troubles

* As explained in the text, what was then described as vitamin B_2 is now recognized to be a complex of vitamins.

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preventing vitamins. Since what had until then been generally called vitamin B (being supplied for example by extracts of yeast) was now found to cure not only *beri-beri* but also *pellagra* it seemed reasonable to call the two factors the first heat-sensitive and the second heat stable vitamin B_1 and vitamin B_2 respectively Vitamin B_2 was thus defined as

the more heat-stable fraction, present for example in heat-treated yeast extracts, and needed for preventing pellagra in man, black-tongue in dogs, and dermatitis in rats, as well as for the growth of rats.

What was not appreciated at the time was that just as vitamin B had turned out to be not single but complex, so in turn vitamin B_2 as here defined would in course of time be found to be a mixture of several vitamins The term was in fact a kind of umbrella covering a variety of things. It would be best if it were now abandoned.

This history has already been outlined in Chapter II but we can here go into a little further detail

TWO OTHER VITAMINS FIRST MISTAKEN FOR THE ANTI-PELLAGRA FACTOR

In 1933–4, some German workers at Heidelberg Messrs Kuhn Gyorgy & Wagner Jauregg were experimenting with a substance called **lactoflavin** (since re-named **riboflavin**, and discussed more fully in Chapter x) This is a yellow colouring matter present in whey meat and other foods and the occurrence of which in milk had been first detected by an English food chemist, Wynter Blyth, as long ago as 1879 The German investigators now found it to help in the growth of rats kept on diets deficient in vitamin B_2 (Fig 27 p 85) Now riboflavin was heat-stable, and it occurred in the extracts used as sources of vitamin B_2 (e g heated yeast extracts) It was natural therefore for Kuhn and his colleagues to consider that they had succeeded in identifying vitamin B_2 and that it consisted simply of riboflavin This would mean that riboflavin as well as being able to promote growth in rats—which it did—should also prevent (1) rat pellagra (2) black-tongue in dogs, and (3) pellagra in humans. This expectation was before long to be falsified.

The next step was in 1934, when Gyorgy now working as an *émigré* from Germany at the laboratory in Cambridge, showed that riboflavin was not in fact effective against the skin disease *rat pellagra* although it helped the rats to *grow* Independently the same conclusion was reached also by Miss Chick, and by myself. The special vitamin which prevented rat pellagra must therefore be something different from riboflavin, although the latter was also a B_2 vitamin in the sense that it was present in yeast extracts was heat-stable and helped rats to grow *Tests on rats proved that these deductions were true.* A rat needed some additional factor to be added to his diet after he had already been supplied with riboflavin if the rat pellagra was to be cured and satisfactory growth ensured. This new factor could be supplied in the form of extracts of various cereals, including maize (Fig 28) György suggested the name **vitamin B_6** for the new rat pellagra vitamin—rather an unsatisfactory name, perhaps because B_6 is a definite enough substance, while the earlier numbers in the series, B_3 B_4 and B_5 were so much more vague and ill-defined

This latter vitamin has since been given the rather better-chosen name of **adermin**, or—the official choice—**pyridoxin** (Chapter x, p 258)

Until the contrary was proved *it had to be assumed* that this heat-resisting vitamin B_6 found in yeast and preventing rat pellagra and needed for the growth of rats was the same as that preventing human pellagra and black-tongue in dogs As Occam had taught in the fourteenth century hypotheses must not be multiplied unnecessarily

THE PELLAGRA VITAMIN DISTINGUISHED FROM THE OTHER TWO

But finally the fact that the true anti-pellagra vitamin was yet a third substance, distinct from either of the vitamins just mentioned, *riboflavin* or the *rat-pellagra factor* (B_6) was proved in some experiments by Birch, György and myself, which we published in 1935 What we showed was that puppies given both riboflavin and vitamin B_6 were still not cured of their black-tongue or able to grow satisfactorily until some further vitamin-like factor was added to their diet The experiments are illustrated in Fig 29 This

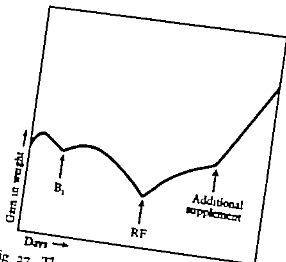


Fig 27 The vitamin activity of riboflavin for rats.

Rats kept on a diet devoid of vitamin-B complex ceased to thrive and soon began to lose weight. They failed to recover completely when *vitamin B_1* alone was given (from first arrow.) The addition of *riboflavin* (from second arrow) improved growth, especially when a further *supplementary extract* thought at that time to contain the hypothetical vitamin B_6 was provided (from third arrow).

Thus, riboflavin was shown to have growth-promoting activity for rats.

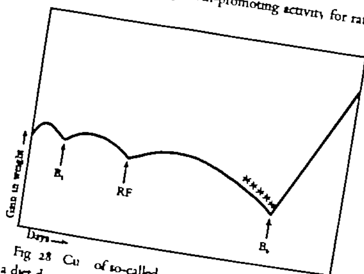


Fig 28 Cu[illegible] of so-called rat pellagra by vitamin B_6

Rats kept on a diet devoid of vitamin-B complex failed to thrive when given *vitamin B_1* (from first arrow) and *riboflavin* (from second [illegible]rrow). Symptoms of dermatitis ('rat pellagra') developed (marked by asterisks) and could only be cured (third arrow) by provision of extracts containing some new additional factor christened *vitamin B_6*

third factor the anti-black tongue vitamin, was the same as the human anti-pellagra factor and also the same as the factor needed for preventing the corresponding disease, monkey pellagra the study of which I had then initiated at Cambridge My colleagues and I pointed out that the third B_2 vitamin the anti-pellagra factor could be distinguished from the other two,

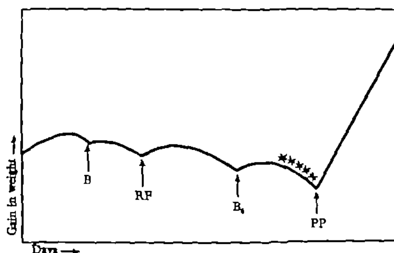


Fig 29 The anti-blacktongue factor for dogs.

Blacktongue in dogs, the equivalent of human pellagra, was not cured by vitamin B_1 or riboflavin, or by extracts containing vitamin B_4 (given from first, second and third arrows, respectively) Its cure needed a fourth factor distinct from any of these (Birch, György & Harris, 1935)

Thus, the existence of a third B_2 vitamin the anti-blacktongue (anti-pellagra) factor different from riboflavin or vitamin B_6 was proved.

TABLE XXVI. *Three components of the vitamin B_2 complex as distinguished by their differences of distribution in foodstuffs*

(Birch, György & Harris, 1935)

	Maize	Fish	Liver extract ('343)	Egg white
(1) Riboflavin	+	o	+	+
(2) Vitamin B_6	+	+	o	o
(3) Pellagra-preventing factor	o	+	+	o

+ indicates that the food is a good source.
o indicates that the food is a poor source.

riboflavin and vitamin B_6 by some striking differences in distribution For example fish contained little riboflavin but plenty of the other two vitamins liver extract little B_6 but much riboflavin and P P factor egg white plenty of riboflavin but little B_6 or P-P factor (Table XXVI)

THE CHICK PELLAGRA VITAMIN ALSO MISNAMED

There is still one last case of a mistaken identity to be mentioned in this long history of disentanglement before we return to the anti-pellagra vitamin itself At the University of Wisconsin in 1935 Dr C. A Elvehjem was working on a disorder in chickens produced by one-sided diets. The leading symptom was an inflammation of the skin and Elvehjem dubbed it chick pellagra believing that it was caused by lack of the same vitamin as that concerned in preventing human pellagra. When some time later the true chemical nature of the P-P vitamin was at last established this assumption was found to be incorrect. The so-called chick-pellagra factor turned out to be something quite different, in fact the same as another vitamin already known to be needed by microbes and previously named **pantothenic acid**.

It will be best to defer to a later chapter near the end of the book (p 258) any further mention of these three new vitamins namely riboflavin vitamin B_6, and pantothenic acid, and return now once again (after this somewhat long but necessary digression) to our more immediate concern, the pellagra-preventing factor

THE TRUE IDENTITY

In 1937 the pellagra-preventing vitamin was finally and at last to be identified as **nicotinamide** The steps preceding this discovery are worth recording as another example of how scientific knowledge grows little by little.

It happens that Funk (who as we recall, had been the first to suggest that pellagra might be a vitamin-deficiency disease) had at one time separated crystals of a substance called **nicotinic acid** from certain vitamin extracts and supposed them to have some vitamin-like properties for his polyneuritic pigeons. However there was no mention of pellagra in this connexion the

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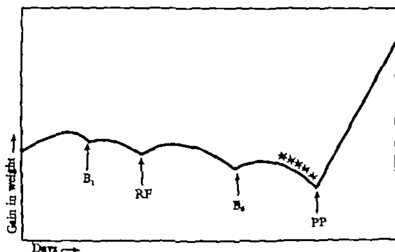


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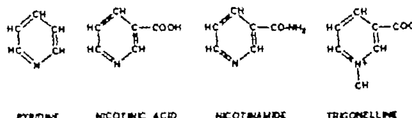
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experiments did not attract much attention and they were forgotten. Later Warburg and Euler both in Scandinavia quite independently studying the chemistry of enzymes and co-enzymes—a group of substances already familiar to us from Chapter III—found that nicotinic acid, in the form of its amide, **nicotinamide** was a component of some of them. Next, tests on micro-organisms by Knight in London and by others indicated that this same substance was a growth-promoting factor for bacteria It was these clues, perhaps, which suggested to R. J Madden, a student of Elvehjem s at Wisconsin, that it might be worth testing the effect of a preparation of the pure chemical, nicotinic acid on their dogs suffering from black-tongue The result was dramatic and unmistakable, and a paper by Drs Elvehjem, Madden Strong & Woolley in 1938 gave the news for which so many investigators were waiting Nicotinic acid, a simple chemical substance of known properties would cure black-tongue Acting on the hint, Miss Chick and Sir Charles Martin found that this substance was equally efficacious in curing pellagra in pigs (a disease which they had recently been studying) and I had as good results on monkeys. And then, almost simultaneously three groups of investigators Fouts and Spies and their respective colleagues in U S.A. and Hassan and I in Egypt, arranged clinical trials to test the efficacy of nicotinic acid in pellagra of human beings. All three found it to be effective, and the reports to that effect were published later in 1937

NICOTINIC ACID AND NICOTINIC AMIDE

Nicotinic acid has perhaps the simplest chemical structure of any of the vitamins Chemically speaking it is a derivative of pyridine, namely pyridine-β-carboxylic acid (Table XXVII) Pyridine, the parent substance is an evil-smelling liquid first obtained about 1851 by the distillation of bone oil or coal tar Nicotinic acid derives its rather surprising name from the circumstance that it happened to be first prepared (in the 1870 s by organic chemists in Germany) by chemical breakdown (oxidation) of the alkaloid *nicotine* present in tobacco Nicotinic acid the vitamin, however bears little or no resemblance in its properties or physiological activities to nicotine! To

TABLE XXVII *Chemical structure of nicotinic acid and some related substances*



remove any possibility that the name *nicotinic acid* might convey the misleading impression that there is some connexion between vitamins and tobacco an American Committee proposed to rename the P-P vitamin *niacin* This alternative name is derived from three syllables in the words nicotinic-acid-vitamin. For a time it became quite widely adopted in the U S A. but has recently been abandoned.

As will be mentioned below it seems certain that the form in which the vitamin functions in the body is not as nicotinic acid itself but as *nicotinic amide* (Table XXVII) When the acid is taken by mouth it becomes converted, partly at any rate to the amide in the body It would in fact be more logical to confer the name of the vitamin not on the nicotinic acid but on the nicotinic amide (or to give its shorter synonym *nicotinamide*)

Indeed when the synthetic vitamin has to be administered, in medical practice, it seems better to give it in its more natural form as **nicotinamide** rather than as nicotinic acid. The latter if administered in large doses can give rise to some slightly unpleasant side effects, such as flushing and tingling in the skin. The free acid is slightly toxic in other words when given in fairly large doses whereas the amide is not

THE NEEDS OF DIFFERENT SPECIES

As has already been mentioned nicotinic acid (or amide) is needed by dogs by monkeys and by pigs all of which species develop characteristic diseases if it is absent from their food Rats however appear to thrive when there



Fig. 29 A. Pig pellagra

Above a pig which had been fed on a diet of maize and casein.
Below the same pig after being cured by nicotinic acid.

is none in their diet, or to speak more precisely they develop the deficiency disease less readily (See below p 96.) Thus the right animal has to be used for biological tests on pellagra

Many micro-organisms also fail to grow if not supplied with nicotinic acid in their culture *medium* A *factor which had previously been named* V factor needed by an influenza organism (*Hæmophilus para-influenzae*) turns out to be the same as nicotinic acid.

ITS MODE OF ACTION

There seems no doubt that the secret of the action of nicotinamide in the body resembling that of vitamin B_1 (p 69) is that it is concerned in the action of certain *co-enzymes* Nicotinamide is a component of two important *co-enzyme systems both of which are concerned in slightly different ways* in oxidative processes in the body namely in the transport of hydrogen atoms. The names of these two co-enzymes are Co-dehydrogenases I and II (or as they are now more often called Co-enzymes I and II or yet another name, the two pyridine nucleotides) Their relationship to each other and structural formulae are given in Tables XXVIII and XXIX. In Tables XXX and XXXI is indicated in merest outline the kind of way in which nicotinamide in the form of these co-enzymes, is thought to be involved in promoting the essential processes of oxidation in the living body

TABLE XXVIII *Two co-enzymes containing nicotinamide (the anti-pellagra vitamin)*

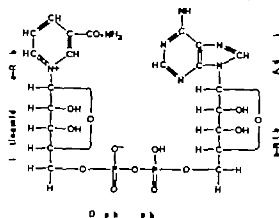
Co-enzyme I = Di-phospho-pyridine nucleotide (nicotinamide + adenine + phosphate 2 ribose)
II Tri- (– + 3)

(For further details of structure see Table XXIX, p 92.)

TESTING FOODSTUFFS FOR THE VITAMIN

For many years the standard method of testing foodstuffs for the P-P factor was to ascertain the minimum amounts which would keep dogs free from signs of black-tongue

TABLE XXIX. *Structural formulae of two co-enzymes containing nicotinamide (anti-pellagra vitamin)*

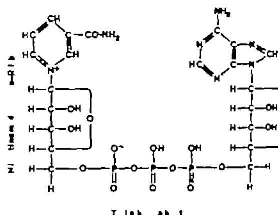


Co-enzyme I

di-phospho-pyridine nucleotide

The structural formula shows that it is built up from the following molecules (left to right)

nicotinamide
ribose
diphosphate
ribose
adenine



Co-enzyme II

tri phospho-pyridine nucleotide

Built up from the same molecules as Co-enzyme I, except for *triphosphate* in place of *diphosphate*

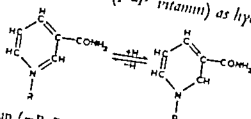
TABLE XXX *The role of nicotinamide as a hydrogen carrier in the living organism*



A simplified scheme (following the explanation of Theorell 1936), showing that *hydrogen* is transported in turn by enzyme systems containing nicotinamide and riboflavin, to be ultimately oxidized to water by oxygen, which is similarly transported by the respiratory pigment, cytochrome.

Thus nicotinamide is a necessary component in the complex chemical machinery concerned with oxidation and respiration in living cells.

TABLE XXXI *Nicotinamide (P-P vitamin) as hydrogen transporter*

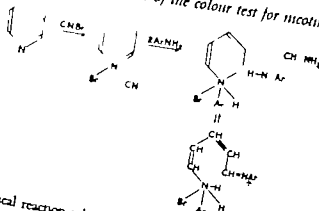


The nicotinamide group (= P P vitamin) present in the molecule of the co-enzymes (Table XXVIII) is able to take up hydrogen from certain substances in the body that can act as hydrogen donators. Then in turn it hands on the hydrogen, being thus itself alternately and reversibly reduced and re-oxidized. This change is associated with an alteration in the valency of the nitrogen atom in the nicotinamide ring system as shown above

As with other vitamins chemical tests are gradually replacing the more laborious biological method of assay

The most usual chemical test depends on the fact that a yellow-coloured product is formed when extracts containing nicotinic acid are treated under appropriate conditions with two reagents in turn viz. *cyanogen bromide* followed by some suitable *aromatic amine* (Table XXXII) Dr Raymond and I found that one of the best aromatic amines to use among the numerous kinds we tried, was one called *para*-amino-aceto-phenone It gave the best

TABLE XXXII *The basis of the colour test for nicotinic acid*



The type of chemical reaction which occurs when a substance containing the pyridine ring structure (e.g. nicotinic acid) reacts with cyanogen bromide followed by an aromatic amine The ring structure is broken and the final product has an intense colour

colour—i e was most sensitive—and was relatively specific—i.e. gave least colour with interfering substances Under suitably controlled circumstances the intensity of the colour in our method of test, increases in proportion to the amount of extra nicotinic acid added to an extract already containing it (Fig 30) and hence an accurate analysis of the foodstuff can be based on this fact.

Other investigators have been busy improving methods for assays by tests on microbes

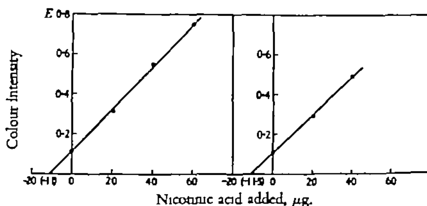


Fig 30 Colour test for nicotinic acid.

By taking known amounts of pure nicotinic acid (20, 40 and 60 microgrammes) and adding them to the extract (bottom scale) the intensity of colour was increased in proportion (vertical scale) Thus, by extrapolation, the amount of nicotinic acid (or its derivatives) originally present in the extract, before the additions, could be calculated. Two duplicate determinations are here shown, giving virtually the same result, viz. 11 or 11 5 microgrammes.

The specimen examined was a sample of urine from a patient with anorexia nervosa.

NICOTINIC ACID IN FOODS

The foodstuffs richest in nicotinic acid (or amide) include yeast, liver and most protein food e.g meat, fish, milk.

The vitamin is not easily destroyed by heat, by acids or alkalis, or by oxidation. Hence it is present in certain canned, processed or cooked foods Canned salmon was in fact used by Goldberger to prevent pellagra in his trials in the field.

HUMAN REQUIREMENTS AND TEST DOSES

It seems that the daily requirement of a human being for nicotinamide is about 10–12 milligrammes per day, that is, about one three thousandth part of an ounce, avoirdupois. With less than 8 milligrammes there is a danger of symptoms of pellagra.

With Dr Raymond, I introduced a method for ascertaining the level of nicotinamide nutrition of human subjects which has been further developed by Dr Kodicek and others. The principle is similar to that for vitamin C (p. 140) and vitamin B_1 (p. 71) and involves giving test doses and ascertaining the amount excreted in the urine. With a person who is deficient, more is taken up by the body than with one who is well nourished, and hence less is lost by excretion in the urine at first—i.e. until the body's needs are met. There is one complicating factor: nicotinamide is partly excreted as such but also partly as certain related substances—classified by the chemist as 'methylated derivatives', e.g. one named *N*-methyl-nicotinamide. Now another such methylated derivative, called trigonelline (Table XXVII), although it has no vitamin activity, happens to occur in certain foodstuffs, e.g. peas and beans, coffee. Hence, to avoid misleading results we had to keep the patient on a diet free from such trigonelline-containing foods for a few hours while the test was in progress. Test doses of nicotinamide (not the acid) are administered by mouth, and we then measure the amount of total nicotinic-like substances, including the methylated derivatives, excreted in the urine.

Little by little, more is being learned about the role of the P-P vitamin in the human body and about the possible effects of minor degrees of deficiency even in the absence of any definite symptoms of pellagra.

TRYPTOPHAN AS VITAMIN PRECURSOR

One point still remains to be mentioned. For some years all attempts to induce in rats a clearly defined deficiency disease provoked by ample lack of P-P vitamin had failed. While diets lacking in nicotinamide readily gave rise to the pellagra-like lesions in dogs, in monkeys or in pigs, as already

colour—i e. was most sensitive—and was relat colour with interfering substances. Under suita the intensity of the colour in our method of te the amount of extra nicotinic acid added to an (Fig 30) and hence an accurate analysis of the f fact.

Other investigators have been busy improvin on microbes.

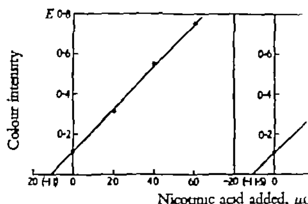


Fig 30. Colour test for nicotinic

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NICOTINIC ACID IN FOOD

The foodstuffs richest in nicotinic acid (or amide) most protein food e.g meat, fish milk.

The vitamin is not easily destroyed by heat, by oxidation. Hence it is present in certain canned, pro Canned salmon was in fact used by Goldberger to trials in the field

body is to a chemist quite remarkable (Table XXXIII) Indeed at first it seemed almost incredible

Another factor which helps to provoke some signs at least of the deficiency disease in rats is the presence in the diet of considerable quantities of maize—that article of food which has always been regarded as pellagra producing *par excellence* We do not yet know for certain what the explanation is It may be the relative shortage of tryptophan in the maize or it may be that the maize contains in addition some toxic factor or anti vitamin antagonizing the nicotinamide

At any rate we can conclude that there are at least two pellagra producing factors as well as a simple absence of the vitamin the first is a deficiency of the protein component tryptophan which acts as a vitamin precursor and the second is the positive action of too much maize added to the diet

CHAPTER V

SCURVY AND VITAMIN C

Probably it is not generally realized that for centuries scurvy was of common occurrence not only among seafarers and explorers and armies in the field but also among large sections of the stay-at home communities in all regions of Northern Europe.

Modern methods of transport were as yet undreamt of and through the dark winter months vegetables and fruits were unobtainable. Freer trade, and the introduction of the potato into Europe have been the two main causes contributing to the decline of scurvy

It is stated that the first distinct account of scurvy dates from the time of the crusades of the thirteenth century although some writers would trace references in writings so remote as those of Hippocrates Indications of the disease are said to have been found in the remains of primitive man An idea of the havoc which it wrought among navigators is conveyed by the few allusions which follow to contemporary records dating from mediaeval times and since

Admiral Sir Richard Hawkins mentioned, in 1593 that 10,000 seamen had died from scurvy within his own personal experience.

On one occasion a Spanish galleon was found adrift at sea, its entire crew dead from scurvy

When Vasco da Gama sailed round the Cape of Good Hope in 1498, 100 of his men perished from scurvy out of a crew of 160

Some of the early records of a cure for scurvy make interesting reading although not as yet of a highly scientific nature

A CURE FOR SCURVY

In Jacques Cartier's second voyage to Newfoundland in 1535 scurvy broke out among his men. Soon 100 out of his 103 men were desperately ill, and twenty-five of them died It is said that in their distress[1] the sailors set up an

[1] From R. H. Major *The Doctor Explains*

SECT XVI



Eing betwixt three or foure degrees of the Equinocti- *The Scurvy.*
all line, my Company within a fewe dayes began to fall
sicke of a disease which Sea-men are wont to call the
Scurvy and seemeth to bee a kinde of dropsie and
raigneth most in this Climate of any that I have
heard or read of in the World though in all Seas it is wont to
helpe and increase the miserie of man it possesseth all those of
which it taketh hold, with a loathsome sloathfulnesse
are they would be content to change their *sleepe and rest* which
is the most pernicious, Because in this sicknesse that is knowne It
bringeth with it a great desire to drinke and causeth a generall
swelling of all parts of the body especially of the legs and gums,
and many times the teeth fall out of the jawes without paine
The signes to know this dise se in the beginning are divers by *The signes.*
the swelling of the gummes, by denting of the flesh of the leggs
with a mans finger the pit remayning without filling up in a
good space Others, show it with their lasinesse Others, com-
plaine of the cricke of the backe &c. all which are for the most
part, certaine tokens of infection.
The cause of this sicknes, some attribute to sloath; some to *The cause.*
conceit and divers men speake diversly that which I have ob-
served is that our Nation is more subje ct unto it then any other
because being bred in a temperate Clymate where the naturall
heate restrayned giveth strength to the stomacke sufficyng it

That which I have seene most fruitfull for this sicknesse is sowre *By sowre Oranges and Lemmons.*
Oranges and Lemmons, and a water which amongst others (for
my particular provision) I carryed to the Sea called Doctor Stevens *By Doctor Stevens water.*
his water of which, for that his vertue was not then well knowne
unto me I carryed but little and it tooke end quickly but gave
health to those that used it.

Fig. 31 Scurvy in the sixteenth century

Sir Richard Hawkins's *Observations in his Voyage to the South Sea* (1593) contains a whole chapter devoted to the problem of scurvy. The extracts given above, it will be noted, include a good account of the symptoms, and a striking reference to the value of sowrer oranges and lemmons

image of Christ upon the shore and prostrated themselves before it in the deep snow chanting litanies and penitential psalms. The pestilence continued unabated however But later one of the sailors learned from the Red Indians that a decoction made from the needles of spruce trees would cure the ail ment. Such a decoction was accordingly made and this time the treatment succeeded. The results were almost miraculous Scurvy disappeared almost immediately A contemporary writer describing the effects of this decoction, said that if all the doctors of Montpellier and Louvain had been there with all the drugs of Alexandria they would not have done so much in a year as that tree did in six days

Two hundred years later in 1734, Bachstrom recorded how a sailor in the Greenland ships was so overrun and disabled with scurvy that his companions put him into a boat and set him on shore, leaving him there to perish without the least expectation of recovery The poor wretch had quite lost the use of his limbs he could only crawl about the ground. This he found covered with a plant which he continually grazing like a beast of the field, plucked up with his teeth. In a short time he was by this means perfectly recovered and upon his returning home it was found to be the herb scurvy grass (as related by Lind)

An important landmark, already referred to in Chapter I is the innovation, introduced by Lancaster in 1601 of supplying fruit juice in the ships of the *East India Company*

A little later observations on scurvy begin to become more scientific in character

EXPERIMENTS ON SAILORS

Lind s famous book on scurvy published in 1753 gave an account of a conclusive experiment which he conducted upon human beings which clearly demonstrated the value of fresh fruit in the treatment of scurvy and the *uselessness of many supposed remedies*

On the 20th May 1747 I took twelve patients in the scurvy on board the Salisbury at sea. Their cases were as similar as I could have them. Two of these were ordered each a quart of *cyder* a-day Two others took *twenty-five gutts* [drops]

en them. Salads of any kind are beneficial; but especially the mild saponaceous herbs, dandelion, sorrel, endive, lettuce, fumitory and purslain. To which may be added, scurvy-grass, cresses, or any of the warmer species of plants, in order to correct the cooling qualities of some of the former; as experience shews the best cures are performed by a due mixture of the hotter and colder vegetables. Summer fruits of all sorts are here in a manner specific, viz. oranges, lemons, citrons, apples, &c. For drink, good sound beer, cyder, or Rhenish wine are to be prescribed.

Thus, we have numberless instances of people, after long voyages, by a vegetable diet and good air, miraculously as it were, recovered from deplorable scurvies, without the assistance of many medicines. For which indeed there is no great occasion, provided the green herbage and fresh broths keep the belly lax, and pass freely by urine, sweat, or perspiration. But when otherwise, it will be necessary to open the belly every other day or so, by a decoction of tamarinds and prunes, adding some diuretic salts; and upon the intermediate days, to sweat the patient in a morning with camphorated boluses of theriac, and warm draughts of *decoct.*

lig.

Fig. 32. Reproduction from Lind's *A Treatise on the Scurvy*, first published in 1753, in which a clear account is given of the dietary cause and cure of scurvy

of *elixir vitriol* three times a-day upon an empty stomach using a gargle strongly acidulated with it for their mouths Two others took two spoonfuls of *vinegar* three times a-day upon an empty stomach having their gruels and their other foods well acidulated with it, as also the gargle for their mouth. Two of the worst patients, with the tendons in the ham rigid (a symptom none of the rest had) were put under a course of *sea-water* Of this they drank half a pint every day and sometimes more or less, as it operated by way of gentle physic. Two others had each *two oranges and one lemon* given them every day These they ate with greediness, at different times, upon an empty stomach. They continued but six days under this course, having consumed the quantity that could be spared. The two remaining patients, took the bigness of a nutmeg three times a-day of an electuary recommended by an hospital-surgeon, made of garlic, mustard-seed, *rad. raphan* balsam of *Peru* and gum myrrh using for common drink, barley water well acidulated with tamarinds by a decoction of which, with the addition of *cremor tartar* they were greatly purged three or four times during the course.

The consequence was, that the most sudden and visible good effects were perceived from the use of the oranges and lemons one of those who had taken them, being at the end of six days fit for duty The other was the best recovered of any in his condition and being now deemed pretty well, was appointed nurse to the rest of the sick.

Some persons cannot be brought to believe that a disease so fatal and dreadful can be prevented or cured by such easy means They would have more faith in some elaborate composition dignified by the title of an antiscorbutic golden elixir or the like Facts are sufficient to convince the unprejudiced. It is no easy matter to root out old prejudices, or overturn opinions which have acquired an establishment by time, custom, and great authorities.

Similar conclusions were reached by physicians in other countries Thus Kramer an Austrian, had written in 1720 If one could only have available a supply of green vegetables or oranges, limes or lemons then one could be in a position to cure this dreadful disease without other help

The advice of Lind, Bachstrom, Kramer and others was acted upon by many explorers. For example, Captain Cook in his celebrated voyage round the world, made his men eat fresh food, both of the animal or vegetable kind whenever possible, and so kept them free from scurvy (Fig 33 pp 104-5) As mentioned already (pp 3 100) the ships of the East India Company were regularly provided with oranges, or lemons, or with lemon juice, from

the year 1601 onwards But over two centuries were to elapse before in 1804, at the instigation of Sir Gilbert Blane, regulations were introduced into the British navy enforcing the consumption of daily rations of lemon juice The result is evident from Table XXXIV

TABLE XXXIV *Scurvy in the Haslar Naval Hospital*

Year	No. of cases of scurvy
1780	1457
1806	1
1807	1

THE LIME AND THE LEMON

Similar regulations were adopted by the Board of Trade in 1865 and for a time there was an end to outbreaks of scurvy in the mercantile marine. An interesting point is that the lemon was often called *lime* wherefore says an American writer British sailors are to-day familiarly known as limies Unfortunately preserved lime juice gradually came to be substituted for the lemon Now preserved lime juice as commonly prepared is of inferior value as an anti-scorbutic, and so doubt was often cast upon the dietary theory of scurvy This misunderstanding concerning the names of the two fruits has been treated historically by Mrs Alice Henderson Smith.

SCURVY TO-DAY

Scurvy is still met with to-day in various remote regions of the globe or among bachelors living alone and catering for themselves, and sometimes in isolated groups and communities It has been more prevalent than is generally realized Outbreaks occurred in all belligerent countries during the First World War (including Great Britain and the U S A) and there were many thousands of cases among the troops on every one of the fronts

In Great Britain during 1917 sporadic outbreaks occurred at Glasgow (where there were fifty cases at a poor law infirmary) and elsewhere in

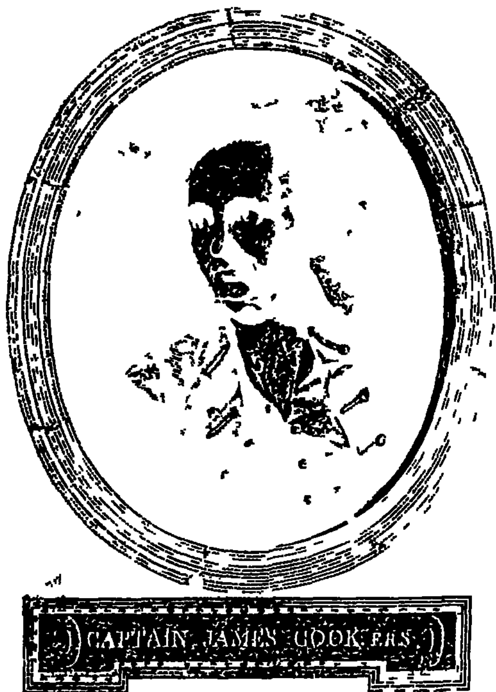


Fig. 33 (1)



(2)



(3)

Fig. 33 (1). Captain Cook, whose *Voyage towards the South Pole and Round the World 1772–5* (the frontispiece of which is reproduced opposite) describes at some length the measures taken to prevent the outbreak of scurvy

He attributes the uncommon good state of health experienced by my people to the extraordinary attention paid by the Admiralty in causing such articles to be put on board as either from experience or suggestion it was judged would tend to preserve the health of the seamen

Sour krout of which we had a large quantity is not only a wholesome vegetable food, but in my judgment highly antiscorbutic, and it spoils not by keeping By this means the disease was prevented getting a foothold in the ship The men at first would not eat it until I put it in practice—*a method I never once knew to fail with seamen for such are the tempers and dispositions of seamen in general that whatever you give them out of the common way although it be ever so much for their good it will not go down and you will hear nothing but murmurings against the man that first invented it but the moment they see their superiors set a value upon it it becomes the finest stuff in the world and the inventor an honest fellow*

Rob of lemon and orange is an antiscorbutic which we were not without. The surgeon made use of it in many cases with great success.

Cook sent an account of these experiences to the Royal Society with the result that, in the words of the contemporary record he was chosen a member of their body and his paper honoured with the prize medal in 1776 (shown above, Figs. (2) and (3))

Scotland (e.g eighty-two cases in a prison camp) at Newcastle (sixteen cases at an infirmary) and at Manchester a national shortage of potatoes was the cause

Measures taken during the Second World War and since to ensure the population s freedom from scurvy are referred to later in this chapter (p 131)

THE SYMPTOMS OF SCURVY

The layman has sometimes been known to confuse scurvy with scurf, with which we need scarcely say it has no connexion ! The characteristic thing about scurvy is the liability for bleeding to occur very easily in different parts of the body (Fig 34) These haemorrhages take their most typical form in the gums which become spongy and enlarged. Their edges become ulcerated, the breath stinking and the teeth loosened

On a diet containing no vitamin C it may take some 4–8 months before these changes in the gums begin to develop At about the same time or perhaps a little before one of the first changes to be noted may be a sallowness of the complexion, and lassitude and mental depression. Together with the bleeding in the gums haemorrhages may also occur elsewhere for example, within the skin or under the skin between the muscles or under the membranes covering the bones (*sub-periosteal haemorrhages*) The haemorrhages may occur either spontaneously or as a result of minor injuries. This leakage of blood from the blood-vessels may be noted either in the form of minute blood-coloured spots under the skin (*petechiae*) or of larger bruises (*ecchymoses*) The blood thus escaping may collect together to form effusions, or perhaps large and painful swellings Hardening and swelling of the calf muscle is a fairly common feature. Sometimes a close inspection by the doctor will reveal small areas of scaliness on the surface of the skin occurring around each small hair on the limb or trunk (*follicular hyperkeratosis*)

As the disease progresses there may be severe anaemia (*the secondary anaemia of scurvy*) causing the noticeable sallowness mentioned above. Another complication, towards the end, is frequently a liability to infection bronchitis, and other respiratory infections particularly are liable to supervene.





(1)

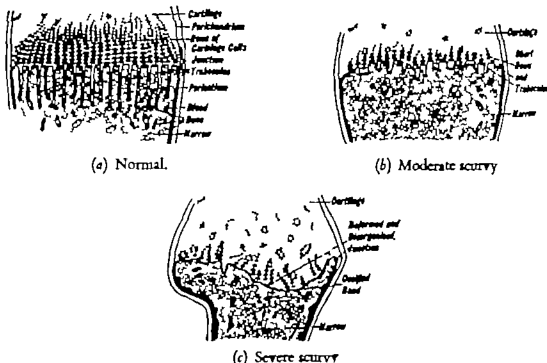


(2)

Fig 34. The consequences of a diet lacking in fresh fruit and vegetables—scurvy in adults.

(1) War-time scurvy in France An example illustrated in the *Paris Medical* in 1917 Note the red spots of effused blood on the legs and the extensive extravasation at the two knees, and (above) the inflammation of the gums.
(2) A typical case of adult scurvy showing the numerous petechiae—spots where blood has effused to the skin.

In scurvy in children an X-ray examination will show that the bones are failing to develop normally There is interference in the formation of new bony tissue—in medical parlance *a cessation of osteogenesis*



(*a*) Normal. (*b*) Moderate scurvy

(*c*) Severe scurvy

Fig 35 Effect of scurvy on bone structure. Diagram of end of rib-bone (after Delf and Tozer, 1918)

SCURVY IN BABIES

At the beginning of this century scurvy had ceased to be the sailors calamity but it was fast becoming a babies calamity Babies were being fed too much on artificially treated and sterilized foods usually of a floury nature. Scurvy is the result of such a diet (Fig 36) This ailment of babies infantile scurvy was first definitely recognized for what it is by Sir Thomas Barlow as recently as 1883 and is therefore often called Barlow s disease. It had become known, rather misleadingly as scurvy rickets and it was Barlow who pointed out that it had no real relation to rickets but was simply scurvy with its own characteristic changes in the bone. The latter had been mistaken

for the bony changes of rickets. To-day it has become the recognized practice to give all bottle-fed babies orange juice or some other suitable carrier of the vitamin (p 153) This is a very necessary precaution to prevent any possibility of scurvy

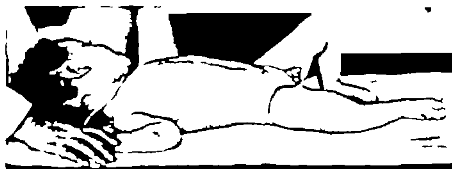


Fig 36. Infantile scurvy ("scurvy rickets" or Barlow's disease). The results of a diet of condensed or sterilized milk and no fruit juice.

The child has swollen painful joints and dreads to be touched. The photograph shows swelling of the right thigh and the characteristic posture (Hess)

SUB-SCURVY

Some investigators have expressed the view that many adults do not take as much fresh fruit and vegetables as they should, and in consequence may be below the standard of vitamin-C sufficiency—not ill with scurvy it is true but nevertheless the victim of very slight or partial or incipient scurvy Perhaps we may agree with this, when we hear that Stefánsson the polar explorer has stated that in his experience the first observable symptoms of scurvy included *laziness gloom and irritability* showing itself in a tendency to *condemnatory and uncalled-for argumentativeness* ! We shall return to the question of sub-scurvy again at the end of the chapter (p 151)

THE DISCOVERY OF EXPERIMENTAL SCURVY

All modern work on scurvy begins with the discovery made by Holst & Fröhch at Christiania (now Oslo) in 1907 that scurvy could be produced in guinea-pigs. After this it became possible to start experiments on the anti-

scurvy vitamin and find out something about its nature and properties, and how it worked Holst & Frölich's own pioneer work in that direction has already been mentioned, in Chapter 1 (p 9)

Scurvy in guinea-pigs is shown in Fig 37 The joints are enlarged and painful, and in the picture the animal may be seen raising its limb off the ground to keep pressure off it or sitting in the face-ache position to ease its inflamed jaw

DOGS CANNOT GET SCURVY

In 1929 May Mellanby found that vitamin C made no difference to the condition of the teeth in her experimental dogs This finding was rather surprising for it was already known that abundance of vitamin C was essential for proper tooth structure and dental health in guinea-pigs. If so why not in dogs? After some experiments we found the explanation to be that dogs do not need any vitamin C in their diet—because they are able to manufacture their own vitamin C in their bodies.

The same is true of many other animal species e.g rats (as had been shown by Helen T Parsons in America) hens and probably most domestic and farm animals. In fact the only species which cannot make their own vitamin C and must therefore be given it in the diet, are so far as our present knowledge goes, man monkeys and guinea-pigs

CONCENTRATING VITAMIN C

Following on the discovery of guinea-pig scurvy in Christiania, attempts were made by several workers to concentrate the vitamin from orange juice or lemon juice and isolate it in a state of purity Two of the principal investigators engaged in this problem were S S Zilva at the Lister Institute in London and C G King in America. The method was to precipitate or extract with various solvents and reagents in turn and test each of the numerous fractions so obtained on guinea-pigs. In 1924 Zilva had obtained a fraction about 300 times as potent as the original lemon juice (see chart, Table XXXV) But for a few years a full stop seemed to have been reached,

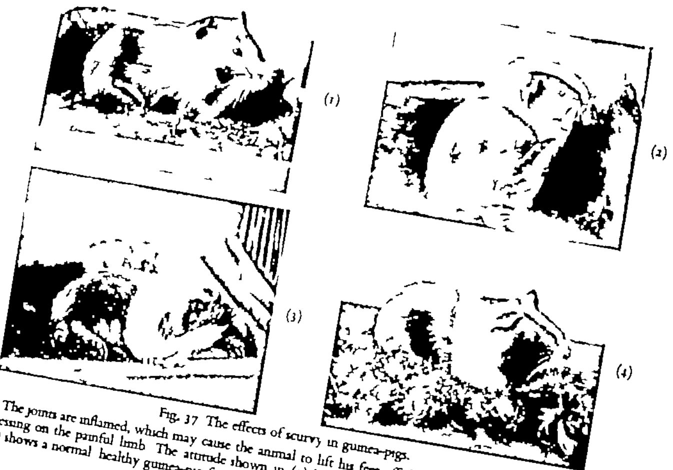
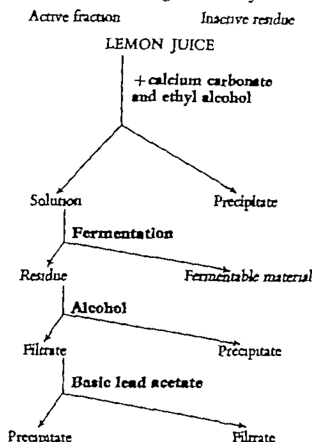


Fig. 37 The effects of scurvy in guinea-pigs.

The joints are inflamed, which may cause the animal to lift his feet off the ground (1 and) to avoid pressing on the painful limb The attitude shown in (3) has been described as the face ache position. (4) shows a normal healthy guinea-pig for comparison (Delf).

TABLE XXXV *Concentrating vitamin C from lemon juice*



little further progress being made Then the solution came quite suddenly and unexpectedly The detailed history is worth recording as illustrating once again how ideas grow gradually and often from different quarters almost simultaneously

HOW VITAMIN C WAS IDENTIFIED

At Frankfurt-on-Main in Germany about 19.8 two chemists at the Public Analyst s Office, Drs Tillmans & Hirsch, had occasion to distinguish between fresh fruit juice and stale fruit juice, or between *natural* fresh fruit juice and *artificial* fruit-juice substitutes. They found they could do this by making use of the fact, earlier noted by Mansfield Clark in America and by Zilva,

that fresh fruit juice bleached a certain well-known dye-stuff ('indophenol oxidation-reduction indicator') Stale fruit juice or artificial substitutes did not do so At the time they thought little more of it and did not at first realize that the bleaching of the dye had anything whatever to do with vitamins. I recall with pleasure, how I came to visit these two German analysts in Frankfurt shortly after this, and how the conversation turned to vitamins They and I had certain common interests quite outside vitamin problems in certain purely chemical or physico-chemical work and I mentioned how I had recently come to desert this chemical work on foods for my newer biochemical studies on the subject of vitamins.

As time went on Tillmans & Hirsch began to realize that the stuff in the fresh fruit which bleached or reduced the dye-stuff and which gradually disappeared from the juice as it got stale had certain features which made it look rather like the anti-scurvy vitamin These two properties, reducing the dye-stuff' and containing vitamin C so often seemed to go hand in hand. Finally Tillmans & Hirsch threw out the suggestion that the reducing substance acting on the dye probably vitamin C, might be the same as a certain reducing substance *already known* to be contained in a number of natural foodstuffs, to wit a so-called HEXURONIC ACID

This substance hexuronic acid ($C_6H_8O_6$) had been isolated from adrenal glands oranges and cabbage several years previously in 1927 by the Hungarian biochemist Dr A Szent-Györgyi working in Cambridge (As a matter of fact the idea that it might be identical with vitamin C had been suggested by myself to our Hungarian colleague when he first obtained his acid but he did not pursue the suggestion further at the time mainly because Zilva had given reasons for supposing that vitamin C itself had nothing to do with reducing properties.[1])

The rest of the story unfolded itself rapidly after this.

Very shortly after Tillmans & Hirsch in Germany had published their theory that vitamin C and the reducing property in foods might be due to

[1] This incident is vividly recalled by Professor King, of Columbia University U.S.A., in an address given, many years afterwards at Edinburgh, in May 1953 (*Proceedings of The Nutrition Society* vol 12, no 3).

nicotinamide was established (Chapter IV) Indeed it was strongly held in some quarters that hexuronic acid could not be the pure vitamin We carried out some tests at the Nutritional Laboratory at Cambridge to try and settle the matter with the result that we were able to satisfy ourselves that hexuronic acid itself was indeed the true vitamin and that in this case for once the vitamin was not an impurity in the crystals

PROVING THE IDENTITY OF A VITAMIN

This was done by showing that whenever there was any hexuronic acid to be found there also was anti-scurvy activity always in exactly the same constant and unchanging amount. One milligramme of hexuronic acid no matter where it was in whichever food it was present, or however it had been treated always had just the same number of units of anti-scurvy activity Thus, some forty different foods were analysed chemically to find the amounts of hexuronic acid in them and in each case the anti-scurvy potency of the food was found to be right for the amount of hexuronic acid in it (Fig 38)

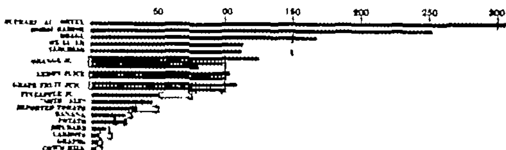


Fig 38 The amount of hexuronic acid in foods is equal to their anti-scurvy potencies.

Lengths of columns represent activities as calculated from hexuronic acid content, estimated *chemically* Cross lines represent values of activities as determined *biologically* i.e by feeding tests on animals (Harris & Ray).

Or again when we *destroyed* the hexuronic acid in a foodstuff by heating it or otherwise we found that the amount destroyed was the same as the amount of vitamin activity which disappeared Similarly when an animal was developing scurvy hexuronic acid was gradually disappearing from the

different parts of its body at just the same rate as these same parts were losing their vitamin value. Or again hexuronic acid could be put through any number of different chemical transformations and operations, and, when eventually recovered again it had the same unchanged vitamin value as originally As a final instance, we may cite the experiments in which we showed that the seed of a plant, known to have no vitamin value, had correspondingly no hexuronic acid in it but once it begins to form shoots (germinates) and the vitamin is born so also the right amount of hexuronic acid is there to account for the newly arrived vitamin These and many similar tests proved beyond doubt that the connexion between vitamin value and hexuronic acid was not any accidental coincidence, but that hexuronic acid was the vitamin itself.

Some years later Szent-Györgyi was awarded the honour of a Nobel Prize, in recognition of his fine achievement in isolating hexuronic acid. As he himself then remarked he had become a father without realizing it—the father of a vitamin

ALTERNATIVE NAMES FOR THE VITAMIN

The name hexuronic acid was never really very well chosen, and it has now been renamed ASCORBIC ACID—to recall its anti-scurvy or anti scorbutic action It is by this name that the pure vitamin C is now generally known.

The story goes that when Szent-Györgyi first isolated hexuronic acid he was at a loss for a suitable name for it Knowing that it was a kind of sugar which means that its chemical name should end in -ose, but being equally ignorant of its exact nature, he very suitably proposed to call it *ignose* The editor of the scientific journal to which the communication was sent for publication suspected a flavour of levity about this name a suspicion in which he was confirmed by his colleagues The editor therefore disqualified the proposed name and asked for some alternative suggestion. Szent-Györgyi replied God-knows

The Americans suggested a new alternative name, ce-vitamic acid instead of the generally accepted ascorbic acid. Ce was to denote the letter C by

which the vitamin had hitherto been known and vitamic acid that the substance was a vitamin possessing acid properties This human propensity for changing names seems well developed, for in the U S A alternative names

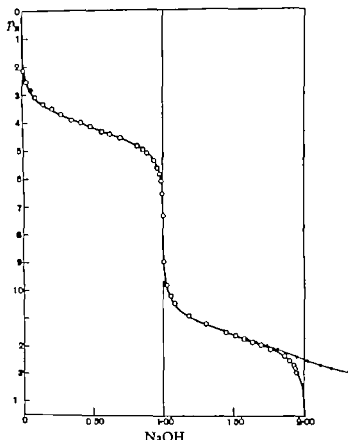


Fig 39 Example of chemical study on a pure isolated vitamin.

Titration curve of pure vitamin C, for determining its ionization constants, number of acid groups, and molecular weight (Birch & Harris 1933).

have been given to other vitamins too Aneurin the name for vitamin B_1 given to it by the Dutch workers who first succeeded in isolating it, has been renamed thiamine in America (p 54) Similarly nicotinamide (p 89) they have renamed niacin amide and adermin has been given the new label pyridoxin In the case of cevitamic acid however the American

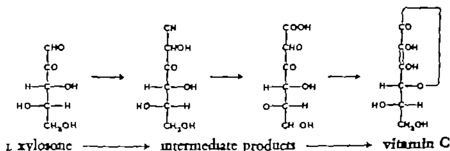
cognomen did not catch on and was dropped after a few years. It is now universally known as ascorbic acid

Before long—and it was considered a remarkable development at the time—ascorbic acid crystals pure vitamin C could be bought in a bottle through any druggist, at so much per gramme, and its chemical properties studied just as exhaustively as those of any other compound, e.g such relatively abstruse things as its titration curve and ionization constants (Fig 39)

SYNTHESIZING A VITAMIN

The final triumph in vitamin chemistry is the artificial manufacture of the vitamin in the chemical laboratory This was accomplished in 1933 almost simultaneously by three Swiss workers named Reichstein Grüssner & Oppenauer and by another team, a group of nine chemists at Birmingham, Drs Hirst & Haworth and their co-workers (Table XXXVI)

TABLE XXXVI. *How vitamin C was synthesized artificially (The last stages only are shown below)*



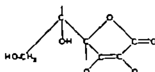
Needless to say it was a complicated undertaking working along gradually in a carefully thought out plan from one substance to another in a long chain as it were until eventually they had the vitamin as their final product. I do not expect my readers to memorize the following list but it suffices to show how many steps were involved before eventually they ended up with the vitamin itself

D-galactose → D-galacturonic acid diacetone → D-galacturonic acid → L-galactonolactone → L-galactonamide → L-lyxose → lyxose-phenylosazone → L-lyxosone (or L xylosone) → ascorbic β-ketonitrile → L-ascorbic acid.

THE VITAMIN FORMULA

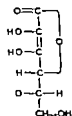
The full structural formula for vitamin C (ascorbic acid) was finally worked out by Dr E. L. Hirst and four collaborators at Birmingham. It is shown below in Table XXXVII which indicates exactly how the atoms of carbon hydrogen and oxygen are arranged in a crystal of vitamin C and to the organic chemist explains some of its special peculiarities In Table XXXVIII it is written out in the more usual way

TABLE XXXVII *Vitamin C Structural model showing actual arrangement of atoms in space*

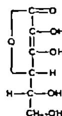


It is remarkable that various compounds differing in only the slightest possible respect from vitamin C in this arrangement of the atoms within the molecule may possess little or no vitamin potency For example the compound so closely related to vitamin C as its mirror image —that is, related as a left-hand glove is to a right-hand glove—is devoid of vitamin activity (Table XXXVIII)

TABLE XXXVIII. *Mirror images Active and inactive forms of ascorbic acid*



L-Ascorbic acid (active)



D-Ascorbic acid (inactive)

THE INSTABILITY OF VITAMIN C

Some of the properties of vitamin C are perhaps worth little more than passing mention here for example its ready solubility in water alcohol, or acetone and its insolubility in fat-solvents the fact that it filters through

parchment i.e. is *dialysable* (as noted in the pioneer experiments of Holst & Frölich) or that, unlike vitamin B_1 it cannot be readily adsorbed (p 56) on fuller's earth, charcoal, etc.

One of the truly notable properties of vitamin C which must be stressed, however is that it is very readily destroyed by oxidation. This destruction is greatly increased as the temperature is raised and also if the acidity of the material containing the vitamin is diminished (or its alkalinity increased) Thus a proportion of the vitamin is lost when vegetables are boiled, and we shall return to this point again later If, however care is taken to exclude all air or oxygen and the reaction kept sufficiently acid it is possible—theoretically at any rate—to prevent destruction. Another factor which favours destruction is the presence of even the slightest traces of the metal, copper

A DESTRUCTIVE FERMENT

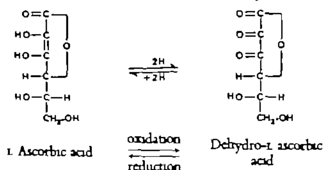
Yet another important cause of loss is traceable to the fact that various vegetables happen to contain inside their cells a ferment, or enzyme, called *ascorbic oxidase* which hastens the oxidation. If this enzyme is by any means set free from within the cells as for example when the vegetable is finely chopped up or when it is heated in warmish water it then proceeds to attack the vitamin without delay and thus the anti-scurvy properties can be lost within a matter of minutes or seconds. Some vegetables or fruits unfortunately contain much of this destructive agency the oxidase such are cauliflower and bananas Others for example black currants contain little or none, and are therefore safe as *stable* sources of the vitamin. Heating to boiling-point destroys the enzyme, although it has the danger of partly destroying the vitamin too if air or oxygen has access

INSTABILITY AND REVERSIBILITY AS SECRET OF *BIOLOGICAL ACTIVITY*

When vitamin C is very gently oxidized by suitable measures a very important product called dehydro-ascorbic acid is formed (Table XXXIX) Its production involves merely the loss of two atoms of hydrogen from the

vitamin Under appropriate conditions as in the animal body this chemical change is reversible that is vitamin C can be formed once again—now by the reverse process of chemical reduction instead of the original oxidation The sequence of changes reversible oxidation followed by reduction can be done in a test tube the gas sulphuretted hydrogen serving to effect the regeneration of the ascorbic acid from dehydroascorbic acid We have reason to believe that this reversible change first the loss of hydrogen and then its recapture, explains in some way the unique properties of the vitamin for preserving the health of the animal organism Its behaviour in this respect recalls that of nicotinic acid (p 93) and riboflavin (p 92) which as we have learned also accounts for their mode of action The chemical role of vitamin C is, however not yet understood to anything like the same degree We shall discuss this point later

TABLE XXXIX *The reversible oxidation of vitamin C*



VITAMIN C AND THE ANALYST

The important work of testing the amounts of vitamins in different foodstuffs in the past meant laborious experiments on animals It was in the case of vitamin C that a chemical method of estimation by titration was for the first time worked out. This, of course, greatly simplifies things. The chemical test which my colleagues and I have elaborated makes use of that same dyestuff which Mansfield-Clark and Zilva had experimented with and which Tillmans had already proposed for analytical use at Frankfurt but the details

of the test have been so arranged as to make it as *specific* for the vitamin as possible—i.e. other things in foods which also bleach the dye are so disposed of as to prevent their interfering To give the details, the principal features of our method of test are as follows First, an extract of the food is prepared in such a way as to avoid loss of the vitamin by oxidation either by air or by traces of copper or more important still by the natural ferment referred to above (ascorbic oxidase) present in so many foods The extracting medium we use is *trichloracetic acid* or better still *metaphosphoric acid* Then it is essential that the actual chemical test itself—i.e the titration of the extract against the dye-stuff—should be done in strongly acid solution and should be completed almost instantaneously If done slowly and in non-acid solution, as Tillmans had tried to do it, many non-specific substances are estimated and not only the vitamin itself—for example the substance, so well known to biochemists, called glutathione—and in consequence the results will be often quite erroneous

The advantage of this chemical, as opposed to the biological method is that a vitamin test can now be finished and the result known in a few minutes instead of taking so many days or weeks Furthermore, a very minute specimen is enough to do a test on—e g 0 03 millilitres of orange juice (a fraction of a drop) suffices instead of the 400 millilitres needed for the old animal test.

With this easy method of testing for the vitamin we have discovered it to be present in a number of places where it was not previously suspected to be The suprarenal glands of animals were already known to contain it but it was surprising to find rat suprarenals were no less than *ten times as potent* as orange juice—which had long been considered the most active known source of vitamin C Tumours also contain a large amount of vitamin C together apparently with certain other rather similar substances and this finding may possibly help to explain some of the special peculiarities of tumours and their fast rate of growth For some reason not yet understood there is also a large amount of the vitamin in the lens of the eye

It has been found that vitamin C is excreted by human beings in their urine in amounts depending on the amount of vitamin eaten in the food

(Fig 40) I have applied this result for diagnosing whether any given ind[ividual] is consuming an adequate amount of vitamin C or whether a chil[d is] suffering from Barlow s disease (see later in this chapter p 140 a[nd] Chapter XIII p 330)

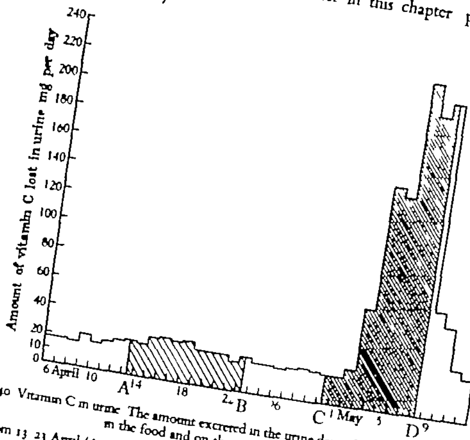


Fig 40 Vitamin C in urine The amount excreted in the urine depends on the amount given in the food and on the past level of intake.

From 13 23 April (A to B) a relatively small addition of vitamin C was made to the diet (about 230 c c. of orange juice taken per day) and as the chart shows there resulted a slight increase in the amount of vitamin C in the urine From 30 April–8 May (C to D), larger doses of vitamin C were given (about 460 c.c of orange juice per day) and it will be seen that there was then a greatly increased output. (Harris & Ray)

In infectious diseases more vitamin C is used up in the body so that less is lost in the urine—unless the intake is suitably augmented with fruit drinks etc (See p 149.)

In various other ways, too this chemical test for vitamin C has been put to good use as in finding the effect of pasteurization on the vitamin value of milk, and in determining the loss during various commercial processes such

as canning preserving and dehydration as well as for checking up the chemical operations leading to the manufacture of vitamin C in its pure crystalline form—an enterprise now undertaken on a large scale by commercial firms interested in the sale of fine chemicals

TESTS WITH GUINEA-PIGS

Before this chemical method had been worked out, the standard procedure for assaying foods for their vitamin-C content had been by means of tests on guinea-pigs The principle was to find the smallest dose of the food in question which when given every day would just suffice to prevent any symptoms of scurvy appearing in the guinea pigs (which were of course fed on a special diet otherwise devoid of any foods containing vitamin C) The test would have to last at least 90 days Or alternatively various graded doses of the food could be administered daily to different sets of guinea-pigs, and again after 90 days the *degree of severity* of the resulting symptoms of scurvy could be assessed. Thus if the symptoms were very severe a score of + + + would be awarded if less severe, + + if slight, + In this way the effect of the given dose of unknown could be compared with that of the known dose of some standard preparation—at first lemon juice.

A rather more accurate, and more objective method (since the subjective impressions of the observer in judging the severity of the symptoms does not then enter into consideration) is a *curative growth test* which I have introduced. This is done in the following way Just before the guinea-pigs are due to begin losing weight from the approach of scurvy graded doses of unknown and of standard are administered and the resulting graded effects on the curves of their recovering growths are noted (Fig 41) Thus standard and unknown can be compared, and the latter expressed in units of the former It is necessary to start dosing your guinea pigs before they have begun to lose weight appreciably otherwise they may have already developed complications in the form of infectious troubles from which it will be too late to cure them even when the vitamin is given.

Yet another method depends on the effect of vitamin C in protecting the

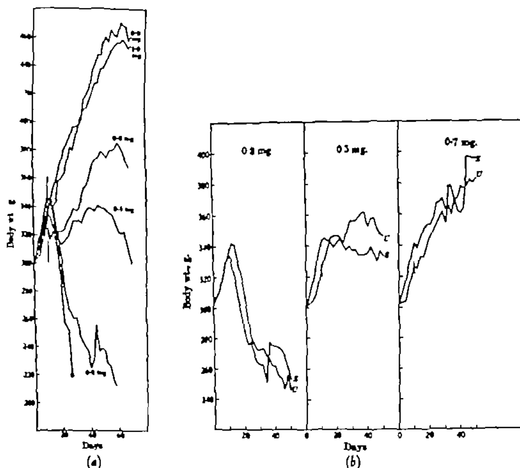


Fig. 41 Assay of vitamin C by curative growth tests.

(a) Guinea-pigs developing scurvy (at dotted line) were given graded allowances of vitamin C, 0–1·0 milligrammes per day They responded by showing corresponding graded degrees of cure as indicated by their graded gains in body weight.

(b) Effect on body weight of graded doses of Standard (S) as compared with Unknown (U), here given at exactly equivalent levels of dosage.

normal structure of the teeth. You administer your graded doses of unknown and of standard in preventive tests for about 15 days. The animals are then killed, and the teeth removed cut in cross-section, and stained and examined under the microscope. The degree of adequacy or inadequacy of the supplements can then be assessed quantitatively The more inadequate the supplement the worse the resulting degeneration of the tooth (Fig 42)

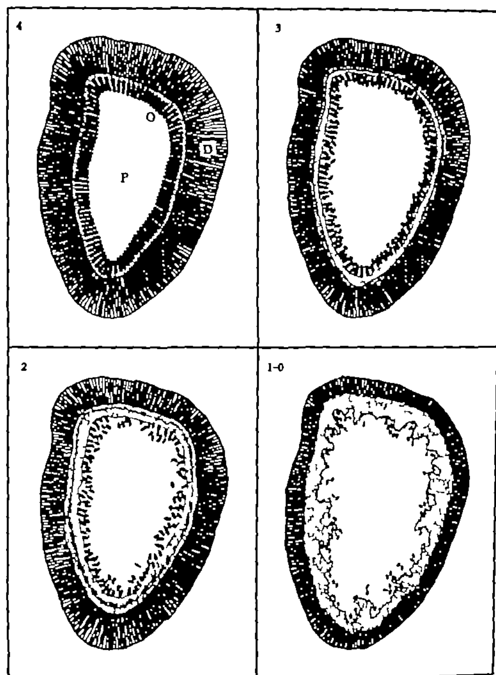


Fig 42. Assay of vitamin C by its effect on tooth structure.

The greater the deficiency of the vitamin, the greater is the degree of degeneration of the tooth structure

The diagrams are purely schematic, and represent the microscopic appearance of cross-sections of the roots of the incisor teeth of guinea-pigs.

Four degrees of protection are shown

4 = Full protection tooth structure normal.

3 = Nearly full protection tooth structure nearly normal.

2 = Less protection tooth structure abnormal.

1–0 = Little or no protection tooth structure very abnormal.

P = pulp O = odontoblast, D = dentine.

CHEMICAL VERSUS BIOLOGICAL METHODS OF TEST

Such biological methods of test are gradually being displaced by the newer chemical methods Indeed almost the only occasion nowadays when one needs to use a biological test is when for some quite special reason the accuracy of the chemical test seems open to question But this is quite a rare event, and in practice the results obtained by the chemical and by the biological methods generally agree remarkably well Some instances of the good agreement which we have found are shown in Table XL. For all the ordinary natural sources of vitamin C, such as raw or cooked fruits and vegetables for jams and for canned fruits and vegetables and most other processed foods the chemical test in its ordinary simple form gives reliable results Only in one or two special cases has the chemical method had to be suitably modified or elaborated in order to deal with certain unusual kinds of products These special cases arise first with caramelized preparations (that is to say foods containing sugar-like substances heated to unusually high temperatures) secondly with certain fermented liquids and thirdly with such manufactured articles as have been treated with sulphite (SO_2) as preservative In these few cases interfering substances are present and suitable means had to be found for removing them or allowing for them

TABLE XL. *Vitamin C in fruits and vegetables Agreement between the results of biological and chemical assays*

Food tested	Biological method used	Ascorbic acid found (mg per 100 g)	
		By biological test	By chemical test
Dried cabbage	Curative growth	463	460
Black currants	Tooth structure	194	237
Black-currant purée	"	138	120
New potatoes	" "	37	35
Cabbage cooking-water	Curative growth	25	25
Old potatoes	Tooth structure	7	9

SPECIFIC CHEMICAL TESTS

Without going into all the technical details it may be worth mentioning how my colleagues and I have been able to do this With the caramelized products referred to (present for example, in dehydrated foods which have been heated unduly) substances called reductones are present which closely resemble vitamin C but have no vitamin activity We can distinguish these from vitamin C by making use of the fact that although both decolorize the

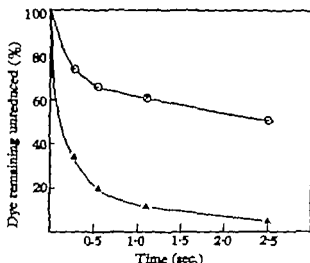


Fig 43 Reaction time curves

Lower curve, vitamin C. Upper curve, gluco-reductone (a substance present in caramelized foods, and liable to interfere in an analysis for vitamin C)

The different speeds at which these two substances react with the indophenol dye enable them to be distinguished from one another

dye in the merest fraction of a second, the precise rates of reaction are somewhat different. By suitably devised electrical machinery capable of estimating accurately intervals of hundredths of a second, the difference can be distinguished and measured (Fig 43) Then again if the product which is being tested is found to have been preserved with sulphite, which interferes in the test (as for example with the preserved fruit pulps used by jam manufacturers) this can be disposed of either by blowing it off in a stream of air or by adding some acetone which removes it. Finally the material to be tested may be so

strongly pigmented, as for example with black-currant juice that we cannot conveniently ascertain in the usual way the amount of dye-stuff which the ascorbic acid is able to bleach since the colour of the dye is masked by the natural colour of the black currant In such an instance we measure the reaction between dye and vitamin not visually but by changes in electro-potential (Fig 44)[1]

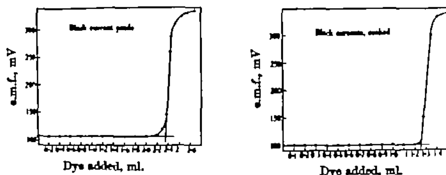


Fig. 44. Analysis for vitamin C by the electro-potential method.

When the titration is complete the e.m.f rises sharply The curves here shown indicate that in the specimen of black-currant purée under examination there were 2 4, and in that of the cooked black currants 1 25 equivalents of vitamin C, respectively

WHICH FOODS CONTAIN VITAMIN C?

We have already said sufficient to indicate—and it has been known for 200 years—that fresh fruits (notably orange juice) and vegetables form the best practical source of the anti-scurvy factor Smaller amounts are to be found in most other *fresh* foods also such as fresh milk meat etc

The best fruits—in descending order—include black currants, strawberries, oranges lemons and grapefruit and the best vegetables, Brussels sprouts, cabbage spinach Some fruits and vegetables are relatively poor such as grapes, plums, pears most varieties of apples blackberries, lettuce Some typical numerical values are entered in Table XLI.

[1] And, to bring the book right up-to-date, the latest method of all (1953) is one employing the new technique of filter paper chromatography invented by my former colleague Dr A. J P Martin, Joint Nobel laureate in chemistry for 1952

TABLE XLI. *Amounts of vitamin C in various foodstuffs*

Food	Amount of vitamin C (milligrammes per 100 grammes) in the fresh, or raw food. Typical values
Fresh fruits, etc.	
Rose hips	1000
Black currants	250–200
Rose-hip syrup	200–175
Papaya	100–50
Guava	100–50
Strawberries	80–60
Orange juice	70–50
Lemon juice	60–40
Grapefruit	45–35
Tomato juice	30–20
Pineapple juice	30
Apples, Cherries, Greengages, Pears, Plums	5–3
Fresh green vegetables, etc.	
Kale	140–120
Brussels sprouts	110–90
Cauliflower	80–60
Cabbage	80–60
Spinach (and other green-leaf vegetables)	70–60
New potatoes	40–30
Turnips	40–20
Fresh green peas	30–20
Lettuce	15–12
Celery, Marrow, Radishes	7–5
Mushrooms	0
Milk	
Human milk	6
Cows milk, untreated	2
Fresh meat	
Liver	Small amounts
Other foods	
Fish, cooked meats, butter cheese, margarine, fish-liver oils, eggs, fat and oils, cereals, bread, yeast, nuts, sugar biscuits, cake, pastry confectionery tea, coffee, cocoa, alcoholic beverages, beef-tea, meat or yeast extracts, condiments	0

Potatoes are not especially rich in vitamin C. They are nevertheless an important source of the vitamin in our national diet—because of the large quantities eaten As might be expected there is much more of the vitamin in new than there is in old potatoes.

Many *dried* foods or *preserved* foods are useless as anti-scorbutics—such as dried peas or beans prunes, dates or figs dried milk corned beef and pressed meats, and so on. But this is not true of certain new types of dehydrated fruits and vegetables prepared by specially devised processes (p 154) *Canned* foods have until recent years generally been reputed to be of little value in this way but in practice there are quite a few exceptions to this rule As Miss Mamie Olliver has shown some types of canned fruits and vegetables may be obtained which are quite effective as sources of the vitamin. The manufacturing conditions have been worked out under which this loss of the vitamin on canning can be reduced to a minimum—as with the more acid fruits, or in the absence of air and freedom from traces of copper in the metal of the can Tinned tomatoes are an example of a product which (as has been known for some time) may be prepared with a high anti-scurvy activity

Canned black-currant juice, or black-currant purée, too formed a valuable addition to the war-time diet during the Second World War it was supplied by the Government for the use of infants and invalids, and was sent to our prisoners of war interned in Germany Another innovation was the use of a syrup made from rose hips It is remarkable that these berries are richer in vitamin C than any other known natural product.

Foods devoid of all vitamin C include cereals eggs and yeast (so valuable for its B vitamins) Again there is no vitamin C in dried wheat, maize, rye, oats or in pulses (beans or peas) although when seedlings of any of these are allowed to sprout the germinating grain and the green shoots do contain the vitamin, as already mentioned in passing

VITAMIN C AND MILK

Cows milk, as Dr Ray and I discovered even when fresh from the cow contains no more than one-third or one-quarter of the vitamin C of human milk (calves do not need vitamin C!) Moreover much of the vitamin C in

cows milk may be destroyed on pasteurization and still more when the milk is left standing Dr S K Kon has even pointed out that exposure of the milk to light as when the bottle is left standing on the door-step has a particularly deleterious effect At any rate we see that by the time the cows milk, after a further heating does eventually reach the bottle-fed baby there is relatively little vitamin C in it compared with that in breast milk. This fact no doubt explains why bottle-fed babies were in the past so prone to scurvy and why special supplements of orange juice or other form of vitamin C are so imperative for them (p 109)

VITAMIN C AND THE COOK

At the Nutritional Laboratory at Cambridge we devoted a good deal of attention, at the beginning of the Second World War to ascertaining in detail how the considerable losses of vitamin C which often occur during the cooking of vegetables and fruits might be diminished. This had become a matter of importance during the war with the cutting down of imports of fruits and vegetables and it was in the public interest that the diminished national resources should be conserved to the utmost and not squandered by inefficient methods of cooking The results of these inquiries were made available to the public in literature issued by the government departments concerned.

Vitamin C can be lost in various ways during the preparation and cooking of food for the table. When for example cabbage is cooked, the destruction of vitamin C by oxidation under the action of heat—a matter already discussed above—is only one cause of loss Another and perhaps even more important cause of loss is that the vitamin C being so soluble, quickly diffuses into the water used for the cooking—which is generally thrown away by the cook, although she might better use it for preparing gravies, sauces and soups The greater the amount of water used for cooking the greater will be the loss by leaching (=soaking out) Then again, if the cabbage is shredded up too finely before cooking there is an increased chance of oxidation of the vitamin especially since the process of chopping or shredding sets free the destructive ferment, ascorbic oxidase which attacks the vitamin, as

already mentioned (p 140) Similarly if the cabbage is put i
brought slowly to the boil instead of being plunged into
water conditions will be ideal for the loss of the vitamin ow
of the ferment The latter is killed by boiling water but it doe
work all the better in warm or hottish water Another serio
is when the cooked vegetable is kept for too long a time on
Mashed potatoes for example fairly quickly lose their vitamin
time the consumer gets them they may contain very little a
showed at Cambridge Finally if excess of sodium bicarbona
used by the cook—she likes it to help her to preserve the gree
her cabbage—there will be undue loss of the vitamin since, as we h
learned destruction is favoured by an alkaline medium A mod
bicarbonate however is not necessarily bad and indeed my
Dr Mapson pointed out that it could be used quite safely for red
excessive acidity and so saving the amount of sugar required in the
of sour fruits say rhubarb (or plums) No harm is done providing
not taken, which is not likely to happen in practice, as it would
colour and the flavour of the fruit.

Tables XLII and XLIII summarize these causes of loss of vitamin
give us simple rules by which the cook may avoid them

TABLE XLII *Causes of loss of vitamin C in vegetables in the kitchen*

(1) Too fine shredding or chopping before cooking
(2) Too much cooking water used
(3) Cooking-water not boiling
(4) Over-cooking
(5) Cooking water not utilized afterwards
(6) Delay on the hot-plate

TABLE XLIII *Rules for avoiding loss of vitamin C in the kitchen*

(1) Avoid undue crushing o chopping before cooking
(2) Use minimum of cooking-water
(3) Start with boiling water
(4) Do not over-cook
(5) Use the cooking water for soups, etc.
(6) Avoid delay on hot-plate before serving

Some characteristic values for vitamin-C losses in cooking are given in Table XLIV

TABLE XLIV *Amount of vitamin C lost by cooking—some typical figures*
(After M. Olliver 1953)

Class of foodstuff	Type of cooking	Percentage of vitamin C		
		Destroyed	Extracted into cooking water	Retained
Green vegetables	Boiling (little water short cooking)	10–15	15–30	55–75
	Boiling (more water longer cooking)	10–15	45–60	25–45
	Steaming	30–40	<10	60–70
	Pressure cooking	20–40	<10	60–80
Root vegetables	Cooked whole or in large pieces			
	Boiling	10–20	15–25	55–75
	Steaming	30–50	<10	50–70
	Pressure cooking	45–55	<10	45–55

DEHYDRATED FOODS

A scientific development during the war-years which attracted more than the ordinary measure of public interest was announced in the summer of 1942. This was the disclosure that research had made it possible to produce a new form of dried food, namely dehydrated fruits and vegetables which for the first time retained virtually the whole of the nutritive value—in particular the scurvy-preventing vitamin—of the raw materials These dehydrated foods were clearly to be of great importance to the fighting services. Dehydrated cabbage or potatoes could now be sent in safety and compressed into an extraordinarily compact and convenient form, to such a place as the Eastern Desert where the fresh vegetables would be quite unobtainable. There they could be reconstituted to make a product which on cooking was almost indistinguishable from the fresh vegetables and retained all their health-giving properties unimpaired.

It is true that dried vegetables had been tried during the First World War

but apart from being unpalatable they were then devoid of vitamin C The importance of the vitamin was not yet fully recognized nor had the means of retaining it during drying been devised Hence troops in that war had developed scurvy when receiving the inferior dried vegetables of those days Naturally enough the attempt to dry vegetables for such purposes was abandoned

The new product which was evolved for the Second World War came as the result of an intensive scheme of research work, carried out under the joint auspices of two government research departments under the Privy Council namely the Department of Scientific and Industrial Research and the Medical Research Council. The Low Temperature Research Station at Cambridge, under the D S.I R., was responsible for elaborating the industrial plant to be used, and the Cambridge Nutritional Laboratory under the Medical Research Council, had the responsibility of ensuring that the processes to be chosen were such as would not cause loss of vitamins especially vitamin C.

FOOD TECHNOLOGY AND VITAMIN C

This latter problem was by no means simple, and much experimentation was necessary before success was to be achieved Among other conditions, it was found that, before drying the vegetable had to be given a preliminary scald in a sulphite bath. This step was necessary in order to eliminate the ferment ascorbic oxidase—which has been so much dogging our footsteps all through this chapter The sulphite likewise inactivates other ferments, which if left would impair the keeping properties of the product and cause it to deteriorate in colour and lose its flavour Then, the conditions of the drying process had to be very closely defined with regard to its temperature, the relative humidity of the atmosphere, and perhaps the exclusion of oxygen. Finally the dehydrated food had to be stored under specially controlled conditions, sometimes in a gas-pack (to exclude oxygen) and again with a low relative humidity—the exact details varying with the product in question.

The saving in transport space is immense (Table XLV) In the case of meat for example, the bones and hides, the hoofs and the offal, can all be left

behind in the meat-producing country The meat itself, after dehydration prior to shipping occupies only a small fraction of the bulk of the raw untreated flesh but even more space is saved in the way of refrigeration plant which in a modern chilled-meat ship takes up so much of the room on board.

TABLE XLV *Dehydrated foods The saving of transport space*

Kind of food	Stowage space needed, cubic feet*	
	For fresh food	For same food after dehydration
Meat, beef	30	11
Egg	91	13
Carrot	385	17
Potato	95	13
Cabbage	1428	46
Fish	333	13

* The figures in this table refer to the number of cubic feet of stowage space needed in order to supply 1 million Calories (food-energy units), in the case of each of the foods mentioned.

It is possible that in the future these dehydrated foods will assume a still greater importance. Seasonal surpluses, and gluts in the market, could be sent to factories to be dehydrated, and so kept for use when wanted. Also products which are common and cheap in one region but rare and a luxury in another could be easily transported in the dehydrated form. Thus in our own dark northern clime we would be able to enjoy the luxuriant fruits of the tropics in the cold days of winter

DOES VITAMIN C OCCUR IN OTHER FORMS?

We can see from Table VI in Chapter II that certain vitamins occur in food-stuffs in various different active forms For example, there are several kinds of vitamin D called D_2 and D_3 etc Again vitamin A may occur in foods both as the coloured pigment, carotene, and also in a colourless form, or as several other closely related substances (Chapter VII) Vitamin B_1 also as we

learned in Chapter III can be present in foods either as the free vitamin or as a combined form that is as co-carboxylase (the vitamin diphosphate) Do similar conditions apply for vitamin C? As far as is known the answer is No Vitamin C in foods occurs in the one form only

It is true that at one time, research workers in Canada advanced the theory that vitamin C in foods existed in a combined form Their evidence was that on cooking the amount of vitamin C in some foods seemed to be increased This was taken to mean that in the raw food a proportion of the vitamin was tied up in some way—that is to say it occurred in a combined form in the raw food so that it could not be detected but that cooking liberated it. We believe, however that the apparent rise on cooking was due to a misunderstanding and could be attributed to the fact that the heating destroyed that oft mentioned ferment, ascorbic oxidase. The figures cited for the foods before cooking had been too low because the ferment present had removed some of the vitamin during the course of the analysis the technique employed failing to take that into account. Then, when the vitamin C *seemed* to be higher after cooking it was merely because there was no longer any oxidase left to attack the vitamin

It has sometimes been supposed, too that there is a great deal of *dehydro-ascorbic acid* (Table XXXIX) in many foods but this seems questionable. We find it in no more than traces in ordinary foodstuffs It is true that it can be detected in products which are undergoing active oxidation but it is extremely unstable and does not stay there for long Its presence, then, is a sign that the vitamin potency is being quickly lost or sometimes that insufficient care has been taken by the analyst to protect the vitamin during the course of the chemical examination

VITAMIN C'S ORIGIN IN NATURE

What is the origin of the vitamin C which is found in most fruits and vegetables—what is its bio-synthesis ? Not a great deal more can be said than what has already been mentioned that dry seeds contain no vitamin C but that when allowed to germinate the vitamin appears. This conclusion could almost be deduced from the experiences of the early navigators they knew

that rations of dried cereals and salt beef caused scurvy but that fresh greenstuffs prevented it. The first to give the matter full scientific proof was Fürth, a colleague of Holst, who was working in 1913 at the same laboratory in Oslo where the pioneer work on scurvy in guinea-pigs was done (pp 109) To prove that vitamin C is synthesized in Nature when a seed germinates he fed guinea-pigs on the seeds (or the seedlings) both before and after germination. His finding was that scurvy was prevented in the latter case but not in the former At the Nutritional Laboratory after we had worked out a chemical test for vitamin C, my collaborator Dr S N Ray was able to investigate the phenomenon more extensively by chemical tests. Similarly he found that a new-laid, fertile hen s egg had no vitamin C in it, but as the embryo developed, he could detect the first appearance of the vitamin.

We do not know definitely from what chemical precursor or precursors, vitamin C is thus produced by Nature in the germinating seedling or in the embryo Nor can we say as yet for certain from what substance the rat or the dog makes its own vitamin C when none is given in its diet. But this should not be more baffling a problem than many already solved, and perhaps the explanation may be ready to tell in some later edition of this book. (Indeed, while this edition is passing through the hands of the printers, Drs F A. Isherwood and L. W Mapson at the Low Temperature Research Station in Cambridge, have been able to trace some of the chemical steps by which the simple sugar glucose, can apparently be transformed into vitamin C, in the living plant, or the rat.)

ULTRA-VIOLET NOT ESSENTIAL FOR SYNTHESIS

Since scurvy in babies is *less* common in summer than in winter the view was at one time held—and was taught in the medical text-books—that it was the ultra-violet rays of the summer sun which caused the synthesis of the vitamin. It happens that this is true for vitamin D—as we shall hear in the next chapter—but not for vitamin C Dr Philip Eggleton and, I in 1925 disproved the theory that light was necessary for the synthesis of vitamin C by some experiments on guinea-pigs We germinated oat-seedlings in the dark, and then fed them to guinea-pigs which we also kept in the dark. Scurvy

was prevented no matter whether our guinea-pigs were in the light or the dark and irrespective of whether the oat-seedlings were etiolated through the absence of light or had the healthy verdure induced by cultivation in the light

We concluded that the reason why scurvy was commoner in winter was not because the ultra-violet rays were poorer but merely that fruit and vegetables are more plentiful in the summer than in the winter

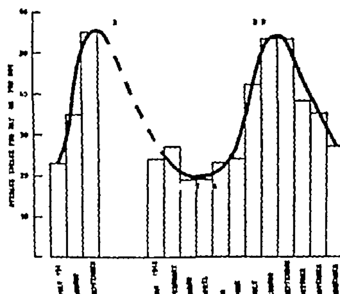


Fig 45 Seasonal curve of intake of vitamin C.

During the summer months the amount of vitamin C consumed in Britain rises as home-produced fruits and vegetables become available, and as new potatoes take the place of old potatoes. The figures recorded in the above curve relate to a study at a boarding school for boys, during the Second World War At that time because of shortage of shipping space no imported fruits or vegetables could be obtained, and hence the vitamin-C intake during the winter was very low during the summer however good use could be made of home-grown garden products.

THE SEASONAL CURVE OF INTAKE

About twenty years later I had occasion with Miss Mamie Olliver to become interested again in this annual periodicity Our purpose was to make a detailed survey of the day-to-day intake of vitamin C by groups of school

children. There was indeed as we found, a most striking seasonal variation. This is seen in Fig 45 The reason clearly is that in the summer garden produce of all kinds is more readily available. By the late winter or early spring the amount procurable has fallen to its lowest ebb This was particularly accentuated during the Second World War by the shortage of imported oranges, tomatoes and the other products on which one relied so much before the war

Only by taking a regular daily helping of potatoes, or better by taking Brussels sprouts or other green vegetables not less than once or twice a week, could one be sure of getting enough vitamin C throughout the winter and spring

ASSESSMENT OF LEVEL OF NUTRITION

If we have reason to think that some people may be at times on diets too low in vitamin C it is useful to be able to detect it. In other words, it is worth while to be able to tell that some particular child, shall we say is so low in vitamin C that he is near the point at which symptoms of scurvy may break out that another child, maybe, is so well nourished in the vitamin that he has enough and to spare and that a third child is in some intermediate category

The test which I worked out for this purpose was the first to be devised for ascertaining the level of nutrition as we may call it, in a particular vitamin Since then my colleagues and I have elaborated similar tests for measuring also the extent of the reserves in other vitamins, e.g in vitamin B_1 and the anti-pellagra vitamin (pp 71 and 95)

The principle is easily understood from Fig 46 The amount of vitamin C in specimens of the subject s urine is measured every day by chemical analysis People whose past diet has been rich in vitamin C excrete on the average more than those whose diet has been poor in it. But such a test of the resting level of excretion is not in itself always a sufficiently accurate method of assessing the nutritional status in the vitamin. What we do in addition is to administer every day a large test-dose of pure vitamin C, and see how much of it is excreted later the same day If the past diet has been

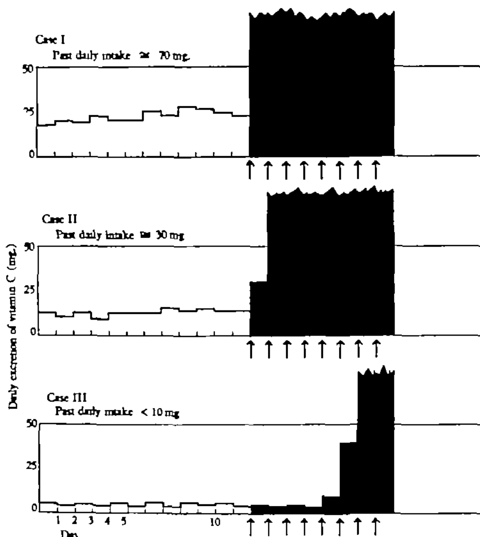


Fig 46. Determining vitamin-C status by the saturation or loading test.

Top middle and bottom panels persons whose past intakes have been high, moderate, and low respectively

The poorer the past intake, the greater the number of days that test doses have to be administered (at arrows) before the body s tissues become saturated and the excess overflows into the urine (black columns)

very well supplied with the vitamin, the body tissues of the patient will b reasonably well saturated with it, and, in consequence, since they are n standing in any need of the extra vitamin administered in the large test-dos most of the surplus will almost immediately flow out in the urine If on t other hand the past intake has been poor the tissues will be relatively u saturated and therefore more in need of the vitamin hence several such dai test-doses will have to be given in turn until perhaps on the fourth or fif day the tissues will be beginning to get saturated, and only then will any lar excretion in the urine be noted. In the extreme case, when the patient is s low in vitamin C that he has already developed scurvy or is about to develo it his degree of *unsaturation* is so great that he will have to receive his dai test-doses for about 7–10 days before his tissues become saturated for the fir time, and the excretion of the surplus begins

DEGREES OF SATURATION

Thus by counting the number of days elapsing before the peak of saturatio is reached, we can assess the level of nutrition of a man or child in vitamin C

A man whose past intake has been very high—say at the level of 75 milligramm per day the U S A. standard of desirable intake —reaches the peak with his ver first dose.

A man receiving still enough but rather less—say 30 mg., the League of Natio Standard of Requirement—reaches the peak on about the first to second day o dosing

But a man suffering from advanced deficiency—ill with scurvy—may take a many as 7–10 days of dosing before he reaches his peak of excretion.

An intermediate number of days, between two and seven, will indicate grade intermediate levels of nutrition in vitamin C.

The diagram (Fig 46) should make all this clear

The test for nutritional status in vitamin C just described is sometimes referred to as the saturation test But this name may be a little misleading because it may perhaps suggest to unthinking people that we want everyone to be completely saturated with vitamin C so overflowing with vitamin C that they can hold no more! Needless to say this is a misunderstanding The

object of the test is to find the degree of saturation or of unsaturation as a means to an end—namely to deduce from it whether the past intake of vitamin C has been high low or indifferent and to put this result on a numerical scale

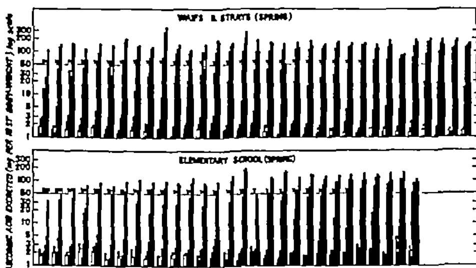


Fig. 47 Saturation test for vitamin-C status on two groups of children.

Above, on a group of 33 boys at a Home for Waifs and Strays below for comparison, on a group of 29 boys at a poor-class elementary school (both in March-April, 1941)

The open columns in the charts represent the amounts of vitamin C excreted on 2 days before the test dosing started the black columns the daily amounts during the test-dosing

A single cross indicates that the response to the test dose was found to be one day late two crosses two days late and so on.

It will be seen that there were more boys in a low state of saturation at the elementary school than at the well-conducted residential home.

VITAMIN C LEVELS OF SCHOOLBOYS IN PEACE AND WAR

It was of interest to compare the vitamin-C status of different groups of school children as determined in this way In tests in 1937 we found that children at elementary schools in poor working-class districts were on the average, lower in their levels of vitamin C than those at a well-managed residential institution—a Home for Waifs and Strays it happened to be in this case. A bird's-eye view of a survey of this kind is seen in graphical form in

the chart of the results in Fig 47 Some of the final conclusions reached in social comparisons of this kind are summarized in Table XLVI.

During the Second World War the levels of the various groups of boys had nearly always dropped as compared with their levels before 1940. The waifs and strays were still better than the boys at the elementary school in a poor district in the same town although both had sunk in proportion below their pre-war position (Fig 48) This fall in vitamin-C status during the war was of course what one was prepared to expect (see p 140) Another point is that levels were better in the summer than in the winter which is also in agreement with previous remarks about seasonal variations in the supply of fresh fruits and vegetables and garden produce (p 139)

In some instances we made a double check, measuring both the day-to-day intakes of vitamin C in the food as eaten over periods of many months and also the levels of nutrition as estimated upon later test-dosing In this way detailed values for *calibrating* or *standardizing* the saturation test were obtained, the known past intakes of vitamin C being correlated with the corresponding observed responses to the test. In the various groups the two went together side by side remaining satisfactorily graded at the various levels (Table XLVII) Hence we could feel content that the saturation test did give a true index of the past intakes [1]

[1] *Other methods of assessing status* Another method of assessing the nutritional level in vitamin C used by some workers, is to measure the concentration of vitamin C in a specimen of the blood. In deficiency (scurvy) it drops to very low levels and when the scurvy is cured it rises to the normal level again. This is a useful test for a check, but probably not so good as the test on the urine for giving one an exact quantitative index of vitamin-C status at various relatively low levels of intake.

At one time a tourniquet test was proposed by a Scandinavian worker named Göthlin. *The principle is to wrap a tourniquet tightly round the arm and then to count the number of* minute haemorrhagic spots—petechiae—which it causes to appear In scurvy as we know the blood is liable to leak out of the blood vessels more easily than normally and therefore there will generally be an increase in the number of such spots. This test, it will be understood, measures the fragility of the capillary blood-vessels. Unfortunately however other things may be involved as well as vitamin C and the capillary-fragility test has not proved a very reliable or specific method of assessing nutritional status in vitamin C.

TABLE XLVI *A war-time survey of the vitamin-C status of different classes of people*

Subjects tested (with notes on dietary regimens)	Whether pre-war or war-time diet, and season	Total no examined	No. found to classify as Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Grade > 5
Boys, good institutional diet								
Unsupplemented	Pre-war winter 1937	25	15	8	2	—	—	—
+Daily orange	1938	29	25	4	—	—	—	—
Unsupplemented	War-time 1940	33	—	5	21	3	1	—
Unsupplemented +15 mg vitamin C daily	War-time summer 1941	10	9	1	—	—	—	—
+25 mg vitamin C daily	"	10	10	—	—	—	—	—
Boys, poor-class elementary school Home diets, unsupplemented		12	12	—	—	—	—	—
Research workers and technical assistants								
Home diets	Pre-war winter 1937	35	10	11	9	5 (Grades 4–5)		—
	War-time 1940	29	1	4	8	11	3	2
Medical students College diets	War-time winter 1940	12	2	3	3	4	—	—
Undergraduates	"	7	1	3		—	1	—
Women's college	summer	16	15	—	1	—	—	—

* Grade 1 denotes the best status, saturating on the first day of test Grade 2 the next best saturating on the second day and so on.

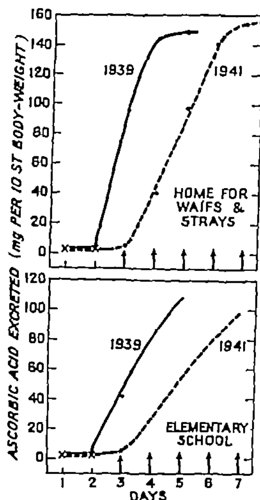


Fig 48 Fall in vitamin-C status as a result of war-time food shortages.

Above are seen the averages of the saturation curves of two classes of boys in April 1941 (broken line) as compared with a similar period in 1939 (continuous line)

It will be noted that both classes had dropped considerably from their pre war position, although the boys at the well-managed residential home still remained at a relatively higher level than those at the elementary school.

(The test-doses of vitamin C were given on the days marked by the arrows.)

GASTRIC DIETS AND VITAMIN C

When my colleagues and I came to examine various groups of in-patients in hospitals by this saturation test we discovered rather to our surprise that those who were lowest in their reserves of vitamin C were mostly people who had been kept on so-called gastric diets By this is meant patients who

TABLE XLVII *The graded relation between the past intake of vitamin C and the time taken to saturate*

Past dietary intake of vitamin C, milligrammes	Time to saturate days
Above 45	1
40-35	1-2
25-20	2-3
Less than 20	3-4

had been suffering from gastric or duodenal ulcers and similar conditions and who in consequence had had to receive special diets free from any coarse residue or roughage, which might irritate the sensitive digestive membranes. Fruits and vegetables are thus generally omitted from diets of this kind The unfortunate result however was that many such people were in consequence having insufficient vitamin C Cases of definite scurvy were even occurring among such hospital patients on gastric diets, as was confirmed by Dr George Graham at St Bartholomew s Hospital, London and by other investigators in other parts of the world. I suggested a name for this newly discovered disease—*scorbutus per culpam medici* !

Happily it is now generally recognized that strained orange juice or ascorbic acid tablets, should be given to people kept on gastric diets and it may be confidently hoped that we have seen the last of iatrogenic scurvy to give it another name.

WOUND HEALING AND VITAMIN C

One trouble experienced by the hospital staffs with these gastric cases kept unwittingly on diets low in vitamin C, was that a surgical wound (after an operation say for gastric ulcer) did not always heal satisfactorily When the error was rectified, and extra vitamin C was given a great improvement in the healing of wounds became apparent. It is now recognized that adequate vitamin C in the diet is a necessity for the satisfactory healing of all kinds of wounds Indeed tests have been made in experiments on guinea-pigs

which show that the *tensile strength* of a wound—that is the pressure at which it breaks—increases in proportion to the amount of vitamin C allowed in the diet.

Fairly early on in the war—around 1940—surgeons were again puzzled to note that healing of wounds in general, including those of war injuries, seemed to be delayed, and not only in gastric cases Dr Paterson Ross, a Professor of Surgery at London University recorded experiences of this kind. After a time it was realized that some of the hospital diets partly because of war-time restrictions were not supplying enough vitamin C. This was put right, and improvements were then reported It has now become the practice in some hospitals to give pre-medication in the form of large doses of vitamin C before surgical operations as a precautionary measure.

LOW RESERVES IN FEVERS

Another unexpected finding emerged as we continued to make surveys for vitamin-C levels by these saturation tests People suffering from infectious ailments we found were generally at low levels compared with healthy people on the same diets It seemed therefore that more vitamin C must be getting used up in the fever or perhaps in combating the infection. This relative shortage of vitamin C was seen in most of the infectious diseases which we studied but to a most notable degree in patients with consumption (*pulmonary tuberculosis*) Fig 49 illustrates this.

We must draw the moral that it is of special importance to give adequate supplies of vitamin C to patients with all acute infections, no less than to those with chronic infective ailments and especially to consumptives A shortage of the vitamin is a double disability Not only is there an additional requirement for the vitamin imposed as we see, by the infection *but any* deficiency may set up a vicious circle, by still further lowering the *powers of resistance* of the patient which it should be the first aim of treatment to restore.

It is interesting to recall that it had long been the practice to give fruit drinks especially orange juice or black-currant syrup to patients with fevers.

This had been done on no very scientific basis but as an empirical measure since it appeared to be beneficial. Quite possibly the new knowledge that the requirements for vitamin C are raised in fever may now supply a rational explanation for the well-established custom.

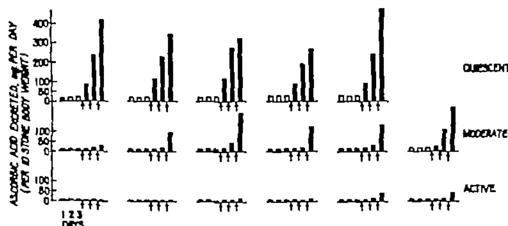


Fig. 49 Lowered vitamin-C status of patients with pulmonary tuberculosis.

Saturation tests on six cases of active T B (bottom row) as compared with six cases of moderate T B (middle row) and six cases of quiescent T.B (top row)

It will be observed that the active cases gave evidence of being in a very low state of saturation (or vitamin-C status), by showing little or no response to three days test-dosing whereas the quiescent cases showed relatively good responses, and the moderate cases intermediate responses.

VITAMIN C AND LIABILITY TO INFECTION

We see that various indications are accumulating to suggest that vitamin C may be concerned in some special way with the powers of the body to counteract infection

First we have the observation, just dealt with, that in infectious disease the usage of the vitamin is increased or the apparent requirement is raised. This seems to imply that reserves of the vitamin in the body may be lowered in infectious diseases. This expectation was found to be justified, since direct tests by my colleagues, Drs Passmore and Pagel, and myself showed that the actual stores of vitamin C in the organs of guinea-pigs were indeed lowered

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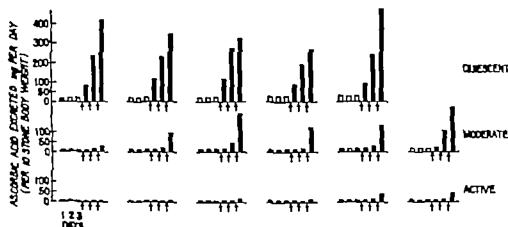


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when they were infected with *Bacterium aertrycke* or *Pasteurella pseudotuberculosis* This was a second piece of evidence

Thirdly we know that guinea pigs, or men suffering from scurvy are particularly prone to become infected with chance micro-organisms—generally in the respiratory tract. (I know it myself from personal experience, *having once been my own guinea-pig in an experiment in which I kept* myself on a scurvy-producing diet.)

The exact meaning of the various observations of this kind is not yet clear By some experts it has been suggested that vitamin C may help in the detoxification processes of the body but the evidence for this has been questioned. One possibility—since vitamin C is needed for the formation of new tissues in general (e.g of blood cells of newly forming bone cells or newly forming tooth cells)—*is that it may help also in the production of certain of the antibodies* concerned in fighting infection. We know that the vitamin is present in very high concentration in certain of the white cells of the blood which are mobilized in some types of infections. This again may give a clue. There the matter must rest, while investigators are actively at work on the problem.

HOSPITAL DIETS

Diet in hospitals often leaves much to be desired Clinicians until recently too often failed to realize that a properly balanced diet is one of the necessary defences in fighting disease. Then again the conditions of cooking and *serving meals in large institutions are often unsatisfactory In a hospital the* food may have to be cooked at a distance from the ward, and sometimes it is necessary to keep it on a hot-plate for a time before *it is served at the bedside* —and we know that any undue use of the hot-plate is a serious cause of loss of vitamin C (p 133)

Since vitamin C as the last few paragraphs have stressed is of special significance in at least three connexions for hospital practice—namely with *gastric diets in infection, and for wound healing*—the need for ensuring a satisfactory supply of it is obvious The importance of the duties of hospital dieticians cannot be overrated in this direction. At some hospitals, these dieticians had already made it a routine procedure before World War II

for every patient to receive the strained juice of one orange each day In war time a supply of home-produced vegetables properly cooked (p 132) became the alternative whenever the patient's condition permitted their consumption Otherwise, the special provision of tablets of ascorbic acid, or vitamin concentrates (black currant or orange) was the substitute

HYPO VITAMINOSIS C

Earlier in this chapter the question was asked whether there was evidence that there was such a thing as sub-scurvy —that is to say that a person might be receiving just enough vitamin C to prevent symptoms of actual scurvy but not enough to prevent some degree of *partial deficiency* of the vitamin in other words of *hypo-vitaminosis*

In attempting to answer this question we may first argue by analogy from experience with animals We have now learned that guinea-pigs may be put on diets low in vitamin C—that is containing just enough to protect them from definite scurvy but not enough to keep them fully healthy The kinds of defects from which they then suffer may be enumerated as follows

(1) poor growth in young animals
(2) abnormalities in tooth structure,
(3) unsatisfactory healing of wounds
(4) irregularity in formation of bone
(5) liability to infection.

It seems probable that similar arguments may apply to human beings. The evidence is not yet conclusive but there are suggestions that some of the troubles listed below may be liable to occur in people on diets unduly low in vitamin C even when scurvy itself is absent. The following are possible effects of partial deficiency of vitamin C in man

(1) poor growth in children,
(2) unsatisfactory healing of wounds
(3) abnormal functioning of the heart
(4) liability to infection.

Two of the items in this list the unsatisfactory healing of wounds and the suggestion of cardiac abnormalities, were among the features studied in a

remarkable experiment on the production of scurvy in a group of human volunteers This experiment was carried out at Sheffield during the Second World War under the supervision of the British Medical Research Council. The experimental subjects were a number of conscientious objectors, who as an alternative to military service, offered to submit themselves to hardship and deprivation in the interests of medical knowledge, and for the alleviation of human suffering In these trials the volunteers were first subjected to an attack of incipient, experimental scurvy by being restricted to a diet devoid of all vitamin C then the disease was cured by the administration of the smallest doses of the vitamin

The object of the experiment was twofold first, to make a thorough study of the various effects of a deficiency secondly to ascertain the amount of vitamin C necessary to maintain health.

THE DAILY REQUIREMENT

This brings us to the matter of the amount of vitamin C needed in our daily diet. The tests on the conscientious objectors at Sheffield, just mentioned, *confirmed earlier supposition that a daily allowance of 30 milligrammes was* enough not only to prevent or cure scurvy but also to provide a reasonable margin of safety By the margin of safety is meant, first, enough to allow for any variation in the individual requirement from one human subject to another (the personal factor) and, secondly enough to prevent *not only* severe deficiency disease—scurvy—but also any minor signs and symptoms attributable to a partial deficiency

This allowance of 30 milligrammes per day is officially recognized as the Standard of Requirement which was fixed by the League of Nations authorities in 1937

VITAMIN C IN PRACTICAL DIETETICS

Enough has already been written in this chapter to indicate what has to be done, in practice in order to ensure that our daily intake of vitamin C is adequate It may however be useful to recapitulate. We may consider in *turn first infants and then adults.*

(1) For infants, we have learned that cows milk is much inferior to human milk in its vitamin-C content and also that it, cows milk is subject to a further loss of the vitamin as a consequence of pasteurization and exposure to light The lesson to draw from this is that orange juice, or other carrier of vitamin C should be given to all babies and particularly to bottle-fed babies. World War II saw the introduction in Britain of the official issues of synthetic ascorbic acid or black-currant juice as substitutes for orange juice, which was then not obtainable Unfortunately not all the mothers took the trouble to draw the supplements to which they were entitled (cf. p 131)

(2) For adults all that need be said here is that the dietician or housewife should aim at supplying one portion at least of fruit or vegetables or of salad per person per day When as during the war-time shortages of 1939–45 diets fell to a low level in their vitamin-C contents during the winter months (p 140) the best sources of the vitamin proved to be (*a*) potatoes (which made up for their relatively poor content of the vitamin by the large quantities consumed) and (*b*) Brussels sprouts

Finally let us recall once again that nursing mothers should be given an extra liberal allowance of the vitamin, and that special care is needed also in the construction of diets for convalescents and for hospital patients, and particularly for those on so-called gastric diets (p 147)

HOW DOES VITAMIN C ACT?

We do not yet know in any detailed way just how vitamin C works It must be supposed, however that its unique action in the body is largely due to the fact, already mentioned, that it is a special kind of *reducing agent*

The animal body is often compared with a furnace Material derived from the food is continually being burned up all over the body in the millions of minute cells of which the body is composed. This gives the necessary heat and energy for the multifarious work of the living being as a whole It is in this process of respiration or oxidation that vitamin C presumably plays a role i.e. in the balanced chemistry of oxidation reduction

This explanation however is not sufficiently full and explicit to satisfy

the curiosity of the professional biochemist, who requires a much more detailed picture of the precise chemical changes brought about by the intervention of vitamin C Future developments may possibly show that the biochemist s answer will be found in the experiments of some American laboratory workers named Lan & Sealock, and Darby and colleagues. They found, in 1944 and 1945 that the vitamin had an effect on one particular aspect of oxidation in the animal tissues namely in the catalytic oxidation of the side chain of the amino-acid tyrosine —*tyrosine* being one of the individual amino-acids from which the *proteins* of our foods and of our tissues are built up So here is one likely clue in reply to the biochemist s query!

EFFECT OF VITAMIN C ON CELL STRUCTURE

Another way of examining the action of vitamin C is to study not the chemical changes it initiates, but the way in which it keeps the cell structures of the body normal, and to ascertain what happens to these structures when the vitamin is lacking

Two answers can be given here The first, due to two American pathologists named Wolbach & Howe, is that vitamin C is needed to enable the cells of the body to produce enough of the normal *cementing substance* or *collagen* to be laid down outside themselves In other words vitamin C promotes the production of intercellular collagen and with a deficiency of vitamin C there is in consequence an inadequate production of the collagen

The second explanation, advanced by the writer of this book and his colleagues makes the answer a little more general It says that vitamin C is needed not only for this particular activity of certain formative cells (in the production of collagen) but that it is needed by the *formative cells of the body in general* to enable them to continue their normal functional activities.

VITAMIN C AND THE STRUCTURE OF TEETH AND BONES

What this means can be understood by considering such formative cells as those in the teeth—namely the *odontoblasts* which are responsible for laying down the hard *dentine* of the teeth or the *ameloblasts* and the *cementoblasts*, the

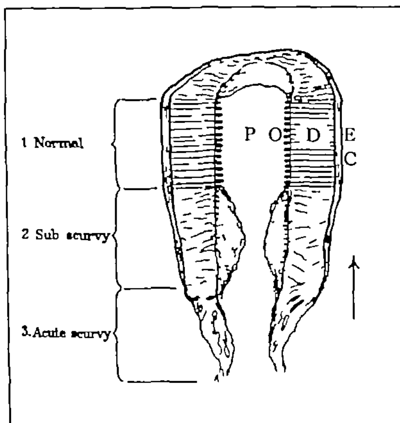


Fig 50 Effects of lack of vitamin C in the teeth.

Highly diagrammatic representation of tooth, split open down middle, showing effects of successive diets.

P = central pulp canal, D = dentine, E = enamel and C = cement.
O are odontoblasts, or dentine-forming cells.

(The tooth of the guinea-pig is growing continuously in the direction of the arrow)

Three periods are shown in turn (1) normal, (2) chronic sub-scurvy and (3) severe scurvy corresponding with these three diets

(1) *Normal* Odontoblasts are seen set out in orderly columns, and the dentine is deposited in regular tubular formation.

(2) *Sub-scurvy* Odontoblasts are partly degenerated, and an irregular formation of dentine results, and a deposit of secondary dentine invades the pulp chamber ("pulp stone)

(3) *Severe scurvy* Odontoblasts are completely degenerated. Little new dentine or enamel can form.

(After Fish & Harris, 1934.)

cells which are concerned in putting down the dense layers of *enamel* and *cementum* respectively around the outside of the teeth With insufficient vitamin C these cells cannot carry on their normal function of building up the new teeth in the growing animal. In consequence, absence of vitamin C means a cessation of the production of these tooth structures the dentine, the enamel and the cementum

With a *partial deficiency* of vitamin C the consequences are, in a way more remarkable. The formative cells suffer some *partial* loss of their normal function and as a result the hard structures of the teeth—the dentine, the enamel and the cementum—are put down in a strangely abnormal form (*Fig* 50)

Similar considerations apply to the effect of deficiency of vitamin C on the structure of bone also—since it is a function of vitamin C to promote the normal formative activity of the *osteoblasts* or bone-forming cells. Hence, in absence of adequate vitamin C production of new bone cannot proceed in a normal fashion (cf p 108)

CHAPTER VI

VITAMIN D AND RICKETS

So far we have been discussing vitamin deficiencies such as *beri-beri* *scurvy* and *pellagra* which may strike the ordinary reader perhaps as rather exotic conditions rarely seen in this country and therefore of little practical concern even to the medical man—let alone the lay person. This criticism is, as it happens a little unjust because scientific research knows no national boundaries and those who take part in it cannot therefore shut their eyes to the fact that in recent years thousands of individuals have died from pellagra, for example, in the U S A. and many thousands from beri-beri in certain parts of the world, while even scurvy has by no means been banished from many isolated areas.

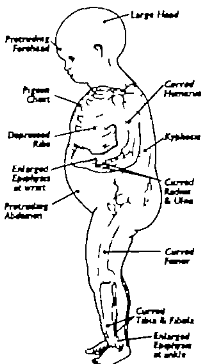


Fig 51 The signs of rickets.

But when we come to rickets we are discussing a familiar and homely subject—at any rate a subject very familiar indeed to our parents for up to our own generation it was impossible to go out into the streets in many industrial areas without encountering children suffering from the crippling deformities brought on by this vitamin deficiency Bow legs, knock-knees swollen joints distorted and weak limbs—such are the results of rickets (see Fig 51) Half a century and less ago rickets of some degree or other of severity was almost universal in some of our big cities and the proportion of definitely *severe* cases, meaning life-long disablement was often quite considerable. Conditions such as those illustrated in Fig 4 (Chap 1) were indeed

so common as to excite no special comment. To-day fortunately cases of rickets so severe as this are relatively rare. This conquest of rickets is one of the major triumphs of modern preventive medicine, and we shall find it of interest to trace the steps by which it has been brought about.

RICKETS THE ENGLISH DISEASE

It was in 1650 that a professor at Cambridge, Francis Glisson, published a treatise on rickets giving for the first time a full description of the disease. The title-page of his book, which was written in Latin, is shown in Fig 52. Slightly earlier still in 1645 a young medical student from Merton College, Oxford, named Daniel Whistler had published at Leyden in the Low Countries an M.D thesis relating to rickets. It bore the title in Latin, *De morbo puerili Anglorum quem patrio idiomate indigenae vocant The Rickets* This is believed to be the earliest written allusion to the disorder

To this day rickets is known in Central Europe as die englische Krankheit although as a matter of fact it has been common in most countries in Northern Europe, and especially in industrial districts but it is fairly rare in Southern Europe, and in the tropics

What is the explanation of this geographical distribution, which has been recognized since the seventies of last century?

RICKETS AND SUNLIGHT

The explanation was given by an English medical man, Dr T A. Palm, in 1890 He it was who first pointed out that rickets is prevalent wherever there is little sunlight and unknown or comparatively rare wherever sunshine is abundant Palm s inquiries were remarkably painstaking For example, he demonstrated that the brilliant sunshine of Egypt was preventive of rickets, for mummified remains were free from all traces of the disorder He was also the first to advocate the use of sunlight as a therapeutic measure in rickets. (The first experimental work on the influence of light was probably that of a Dr J Raczynski, who showed in 1912 that if he exposed puppies to sunlight



Fig 52 Title-page of a seventeenth-century work on rickets.

Francis Glisson's pioneer treatise was first published in Latin in 1650 The third edition, published in Leyden in 1671 contains the well-known title-page reproduced above It depicts a physician in his consulting room examining a patient suffering from rickets. On the wall are seen specimens of a bowed femur and a curved spine

he was able to increase the amount of mineral substance in their bones he concluded it is the sun which plays the principal role in the etiology of rickets)

It is therefore roughly true to say that a map of the incidence of rickets is the practical equivalent of a map of the deficiency of sunlight But why does sunlight prevent rickets in this way? The reason, as we now know is that the ultra-violet rays of the sun are able to synthesize the anti-rickets vitamin in the body even when none is given as such in the food. But many years were to elapse before this explanation was to be found.

RICKETS AND PURDAH

Our generalization above about sunlight and rickets we said was roughly true Why this reservation? Because sometimes rickets still develops in the sunniest climates if social conditions or personal habits tend to rob the individual of his place in the sun Thus in certain parts of Italy where the sun is bright but the people exist in miserably overcrowded quarters in narrow sunless streets, rickets has been of common occurrence and the same is equally true of parts of Turkey and the Levant. In Egypt, too rickets has been reported among certain classes and a recent writer has said if the child sees the sun at all he is fortunate, and as to playing in the open air or bathing in the sunshine, his mother would consider such a suggestion madness Similarly in Algiers the land of sunshine rickets has been frequent in the cities the infants are breast-fed, but the rite of *purdah* is practised. Compare this with an account from Kashmir In Kashmir where osteomalacia is very common among the *purdah* women rickets is hardly ever seen because the children go out from infancy and are in the sunlight all day Soon after birth they may be seen sitting astride the mother s or father s hip with no clothing other than a cap

The *purdah* system and similar social or religious customs by which women and children are confined indoors in many Eastern countries must be held responsible for a vast amount of avoidable disease. Osteomalacia, the adult equivalent of rickets has been only too prevalent in many parts of India in

China and Japan, in Bosnia, in Egypt, and elsewhere. In Northern China a few years ago it was said that 2–5 per cent of the pregnant women there died in labour as a consequence of the disease.

RICKETS AND SMOKE ABATEMENT

The active rickets-preventing rays are the *shorter waves* of the solar spectrum (the so-called ultra violet) which in industrial districts are so largely shut out as a result of the pollution of the air by the factory or the domestic chimney. This explains why rickets is more prevalent in industrial than urban districts. Here is a strong plank for the platform of the smoke-abatement reformer!

Rickets also has a seasonal variation, being most prevalent after the dark winter months. Now that we know of the connexion between rickets and sunlight the explanation is self apparent.

DIET AND RICKETS

There is, however, another factor as well as sunlight in the prevention of rickets.

The children of the Eskimos, for example, although they spend their days in dark huts and the arctic night lasts half a year, remain free from rickets. Why? The explanation is that the Eskimos (and most inhabitants of Scandinavia) eat ample amounts of fatty fish including fish-liver oils. Such food contains sufficient anti-rickets vitamin to afford them ample protection, light or no light.

In industrial districts in England the severity of rickets was undoubtedly largely accentuated by the use of unsuitable food. In the South London slums Dr Corry Mann found in 192[illegible] that the disease was most prevalent and most severe among people on the border line of poverty who chose a cheap diet for their infants, generally sweetened condensed milk.

It is of interest, too, to recall that as long ago as 190[illegible] a Leeds physician, Dr W. Hall, pointed out that rickets was much less prevalent among the Jewish than among the Gentile families in the neighbourhood, a

circumstance which could be attributed to the better dietary habits of the former Here are his statistics (Table XLVIII)

TABLE XLVIII *Rickets among Jews and non-Jews at Leeds* (Hall, 1902)

		Rickets (%)	Faulty teeth (%)
Good district	Jewish school	5	11
	Gentile school	8	38
Poor district	Jewish school	7	25
	Gentile school	50	60

IS RICKETS STILL COMMON?

Although we have records showing that rickets must have been common at least thirty years before Glisson s treatise of 1650 (and no one knows how long before that) few attempts were made to estimate exactly how prevalent it was until about 1870 when Gee concluded that roughly one-third of the children of London were suffering from what we should now call *severe* rickets and similar figures were found by Dr Ritchie for Manchester at about the same date

Coming to later times we find from a government report published in 1920 that among 16 000 cases of crippling in elementary school children, 11 *per cent (i e 1760 cases) were due to rickets*

With the growth of the infant welfare movement, the advocacy of sounder dietaries and the routine administration of cod-liver oil (or more recently halibut-liver oil or synthetic vitamin-D preparations) to young children an unmistakable improvement is apparent. The crippling deformities seen not infrequently at the beginning of the century are now almost unknown and there is a steady decrease in the number of cases of moderate severity

Now turn for a moment to the figures given in Table XLIX, which show *inter alia* that according to the *official report to the Ministry of Health* in 1928 an examination recently carried out in London schools of 1635 un-

selected five-year-old school children revealed the presence of rickets [or rather evidence of past rickets] in 87 per cent.

Nor was this all. We had authoritative pronouncements issued no longer to support the statement that rickets still remained the *commonest cause of crippling* in childhood in this country and was also the commonest cause of difficulty in childbirth occurring among women later in life. The latter trouble was a legacy of the malformation of the bones of the pelvis caused by rickets in the mother's childhood.

To-day no child *need* have rickets.

TABLE XLIX *Estimates of prevalence of rickets among school children*

Date	Place	Incidence of rickets	Authority
1868	London	33% with severe rickets	Gee
1871	Manchester	33%	Ritchie
1915	L.C.C.	80% with some degree of rickets	Dick (1600 children examined)
1920	Durham	82%	McIntosh (1300 children examined)
1926	England and Wales	50% " "	Ministry of Health (1000 children examined)
1928	L.C.C.	87% " "	Newman (1600 children examined)
1933	Woolwich	30%	Medical Officer of Health (1440 children examined)
1933	Manchester	20–43 "	Chisholm
1935	Leeds	30% " "	School Medical Officer

A WAR TIME SURVEY

One of the latest of the large-scale national surveys on rickets was that carried out by the British Paediatric Association in 1944 at the suggestion of the British Ministry of Health. Its object was to ascertain whether there had been any increase in rickets as a result of the war and of the consequent food shortages. No less than 5283 young children were examined in this survey

they were all between the ages of 3 months and 18 months and they resided in twenty-three different areas in Great Britain and Ireland. The conclusion finally reached was that the incidence of rickets diagnosed radiologically in children between 3 and 18 months of age is

2½ per cent before 6 months and
4 in the first year

This meant that about 24 000 children in Great Britain still suffered from rickets every year! A more reassuring finding was that there was only a negligible amount of rickets in children of over a year old.

Certainly these statistics compare most favourably with those of earlier years but there is no room yet for complacency

HOW RICKETS CAN BE PREVENTED OR CURED

The figures cited in Table XLIX could be duplicated from other countries. In America, for example Drs A F Hess & L. J Unger estimated in 1922 that at least 50–75 per cent of artificially fed children in the large cities had rickets.

A woman physician Dr M. M. Eliot showed in 1925 and 1926 that at New Haven, Connecticut U S.A. some degree of rickets was practically universal—namely it occurred in no less than 96 per cent of the infants examined But the most instructive of her findings was that in the children not receiving special treatment, moderate or severe rickets almost always resulted, while in the group to which suitable medical treatment, either cod-liver oil, sunlight or ultra-violet irradiation was administered, there was no *severe rickets and only very little moderate rickets*

In a similar way in 1924 Dr Gebhart working on behalf of the New York Association for Improving the Condition of the Poor in the Italian Quarter where rickets was nearly universal, was able to keep at least 70 per cent of the babies free from rickets by giving them cod-liver oil during the winter months.

CURES FOR RICKETS

The oldest remedy for rickets is cod-liver oil.

It appears that the virtues of cod liver oil have been popularly appreciated in the continent of Europe along the shores of the Baltic and the North Sea for many centuries The earliest medical record of which we have any knowledge refers to its introduction into the Manchester Infirmary in 1766 for chronic rheumatism while in 1848 it was prescribed there for *malacosteon* or adult rickets Outside Manchester medical men appear to have remained unaware of the remedy for a generation or two It was introduced into the continent *via* Holland in 182[illegible] and spread from there to France and to Germany But it will surprise us to learn that up to about 1922 some of the most prominent baby specialists and pharmacologists in England, Germany and U S.A. still remained entirely sceptical about its value in rickets supposing it to have no special nutritional advantage over any other easily digested oil or to be merely an accessory nerve food !

In fact, the clinicians were not fully convinced and cod-liver oil did not come into its own until the research workers had demonstrated in the laboratory that rickets was a vitamin-deficiency disease and that cod-liver oil was an exceptionally rich source of the anti-rickets vitamin.

NEWER REMEDIES

More modern still is the use of certain other fish-liver oils such as halibut- or tunny liver oils These have the advantage over cod-liver oil that they contain a higher concentration of the vitamin. Therefore the dose is only a few drops instead of two or three teaspoonfuls—an amount which was sometimes nauseating or occasionally caused digestive upset These new alternatives to cod-liver oil are of quite recent introduction, following on discoveries in vitamin history which we have still to discuss But we may say at the moment, that repeated observations on human beings have proved beyond any doubt that rickets can invariably be prevented or cured whenever enough vitamin D is added to the diet, provided the diet itself is not grossly improper

in other ways e.g in its content of mineral salts. Those who like statistical evidence of such claims will find it in Tables L and LI.

TABLE L *Proof that rickets is preventable Necessity for adequate allowance of vitamin D*

(From the data of J H. Hess *et al* 1930)

Method of treatment	No of infants	Result after 5–8 months	
		No of cases of severe rickets	No of cases of slight rickets
No treatment	34	9	10
Cod-liver oil (1–3 teaspoonfuls to 2 tablespoonfuls)	44	2	4
Artificial vitamin D (10 drops of 100 D)	6	0	6
Artificial vitamin D (10–20 drops of 100 D)	63	0	0

TABLE LI. *Tests of effectiveness of alternative forms of anti-rickets treatment Results on negro infants*

(Selkirk *et al.*, 1928)

Method of treatment	No of infants	No of cases of rickets	No. of cases of rickets (%)
Untreated	22	17	77
Cod-liver oil	45	20	44
Irradiation + cod-liver oil	43	9	21

IS IT NATURAL?

Some of my readers may be getting restless I am preaching that in our northern climate it is necessary to supplement the baby's bottle with extra vitamin D (fish-liver oil or artificially prepared synthetic vitamin D) in order to prevent rickets I am met with a chorus of criticism—Can you not rely on nature's food? What you suggest is unnatural How did our

ancestors get on before this new fangled doctrine about vitamins had been heard of?

My rejoinder as to its not being natural is to ask the question How far is it natural to live in this sunless climate of ours? In more natural sunnier climates such treatment would not be necessary And how much of our life—our habits of clothing shelter artificial heating and in fact the whole complex fabric of our artificial civilization with its incessant interference with primitive behaviour—is natural ? As to the argument about our ancestors the reply is that they did have rickets in these regions of the globe and in any case the death rate of infants was so unbelievably high—see Chapter XIII—that our ancestors are no authority to appeal to far from it! And as to relying on natural foods —clinical experience and chemical tests both go to show that there is relatively little of the anti-rickets vitamin in most natural foods. Hence the need for supplementing it—unless of course, you are fortunate enough to be able to bask in nature's sun

THE CHEMISTRY OF RICKETS

We have already hinted that rickets is due to insufficient mineral matter being deposited in the bones This causes them to become soft and so to bend. It is particularly the growing part of the newly forming bone which fails to become mineralized properly e g the ends of the long bones of the arm and leg The medical man can diagnose or detect rickets therefore, by taking an X-ray picture, and seeing that there is insufficient density at the end of such bones, e.g at the end of the forearm by the wrist. He can also tell when rickets is healed by seeing that the end of the bone has become properly mineralized and dense again The difference is shown up clearly in Fig 53

Another interesting point is that in rickets the *blood* is short of these mineral constituents (viz. phosphate and sometimes also calcium) In fact it is my belief that this is the direct reason why the bones fail to mineralize. Hence our pathologist has another way of diagnosing rickets in addition to the X-ray method that is by an analysis of the blood When the rickets is being cured the blood becomes normal again. (Another and more recent method

depends on a measurement of the amount of the enzyme (ferment) *phosphatase* present in the blood. This is normally a constituent of the newly forming part of the bone, but in rickets, when this area of the bone remains soft and overgrown, the enzyme leaks out into the blood in increased amounts As the bone becomes healed, less phosphatase is found in the blood again.)

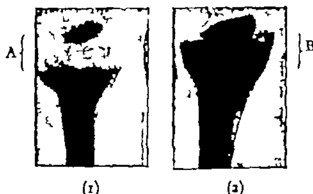


Fig 53 X-ray photograph of rickets and cure. (1) Severe rickets. (2) Healing in progress, under treatment by ultra-violet rays.

The X-ray is from the end of the forearm, near the wrist (end of radius) The absence of properly calcified, newly forming bone is well apparent in (1), and the formation of good new bone with cure, in (2) (cf. appearances at A and B)

To the pathologist the characteristic picture is that seen at A—described as an overgrowth of *uncalcified osteoid tissue* (so-called *rachitic metaphysis*) in place of *normally calcifying newly forming bone* at the growing end of the shaft of the long bone (*epiphyseal end of diaphysis*).

CALCIUM AND CONVULSIONS

A variety of convulsions met with in infancy called nutritional tetany has long been known to be of common occurrence in rickety or malnourished babies The reason is this same change in the blood chemistry just alluded to It is specifically the shortage of *calcium* in the blood which makes the muscles and nerves supersensitive hence they start twitching too readily In other words the baby has convulsions. Cure the rickety condition by giving vitamin D the concentration of calcium in the blood is increased to normal and the convulsions disappear But in rickets more often than not it is the phosphate, rather than the calcium, which is low This explains why one may often have rickets without concurrent tetany



Fig 54. Osteomalacia rickets in adult. Such cases, dubbed marmalade legs were seen in Central Europe after the First World War

RICKETS AND WAR

There is also juvenile, or adolescent rickets which is very much less common than true rickets (the latter occurring almost always within the age limits of nine months to two years when the baby's bones are growing particularly fast) Juvenile rickets was quite common, however in Central Europe at the

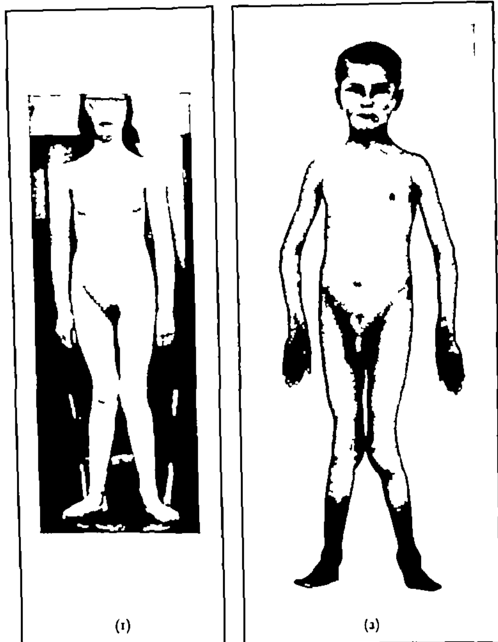


Fig 55 Juvenile rickets.

(1) A case in a girl aged 14½
(2) A case in a boy aged 15
Note the knock-knees and thickened epiphyses at wrists and ankles.

close of the First World War in consequence of the breakdown of food supplies. Some bad cases are shown in Fig 55 In the East it is still not infrequent *purdah* again being to blame.

RICKETS IN DOGS AND RATS AND THE DISCOVERY OF AN ANTI-RICKETS VITAMIN

As with other vitamins it was experimental work on animals that made possible our later advances in knowledge, with all the new power it has given us of preventing disease. Our understanding of the nature of rickets and of the properties of the anti-rickets vitamin is based very largely on such animal experiments



Fig 56 Dog with rickets (Mellanby).

Once again it is a story of slow development. Most current writers overlook the fact that a series of half-forgotten early pioneers succeeded in producing rickets experimentally beginning as long ago as from 1838 and continuing to 1909 [1] Certainly these workers had no proper appreciation of the precise nature of rickets They focused their attention almost entirely on

[1] Early experiments on rickets in dogs were carried out by Guerin (1838) Voit (1880) Baginsky (1882) Stilling & von Mering (1899) Aron & Sebauer (1908) Dibbelt (1909)

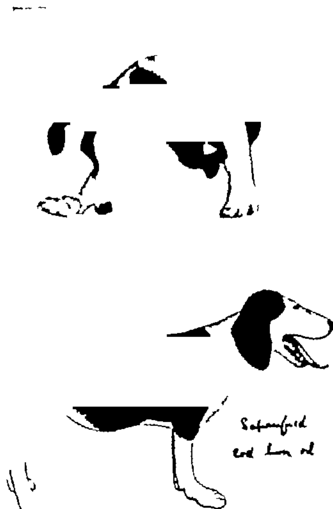


Fig 5 Two puppies from the same litter both given an identical diet except that the one below was given the *addition of a trace of a vitamin-D concentrate* and remained normal while the one above had none and so developed rickets (Steenbock)

the importance of lime (calcium) in preventing rickets During the later half of the last century however the virtues of cod liver oil as an antidote for rickets in human beings had become widely recognized and in 1889 the London surgeon Bland Sutton, carried out some experiments at the Zoo on rickets in monkeys lions bears and birds, and demonstrated the remarkable value of cod-liver oil as an antidote

By 1906 Hopkins could refer to rickets as a disease of which we have had for long years knowledge of a dietetic factor the real error being to this day quite obscure although certainly of the kind which comprises these minimal qualitative factors [1] The vitamin theory was by this time gradually developing for already in 1897 Eijkman in Java had discovered experimental beri-beri which he was soon afterwards (following Grijns) to recognize as a deficiency disease while in 1912 Holst & Frölich in Christiania similarly recognized scurvy to be a deficiency disease and Funk in 1912 propounded the theory that rickets was also due to absence of a specific vitamine the anti-rickets vitamine [1]

At this stage, following the discovery by the Americans McCollum & Davis in 1915 that there were separate fat-soluble and water-soluble factors A and B [1] Edward Mellanby then at Cambridge, undertook a detailed investigation of the various dietary factors which conduce to rickets in puppies Starting *de novo* he examined the influence of the several known vitamins and various other food constituents (1919 1921) He found that cod liver oil and other animal fats already known to contain the fat-soluble A of McCollum & Davis protected his puppies against rickets whereas various vegetable fats did not do so This seemed to lend strong support to the identification of the anti-rickets vitamin with fat-soluble A It proved definitely the existence of the dietetic factor in rickets

Slightly later also in 1921 two independent papers by two different groups of Americans (Professors Sherman and McCollum and their respective co-workers of the Universities of Columbia, New York, and Johns Hopkins, Baltimore) described for the first time how rickets could be produced also in rats. These American workers drew attention to the fact that the amount and the proportion of the *mineral elements* calcium and phosphate in the diet also was of great significance, no less than the supply of the anti-rickets vitamin. This was especially true for the production of rickets in rats [2]

[1] See Chapters I and II.

[2] To produce rickets in puppies it was sufficient to deprive them of vitamin D whereas to produce rickets in rats—at any rate of any degree of severity—it was necessary to interfere with their mineral allowance as well. This was found to be best done by giving a relative excess of calcium and a deficiency of phosphate. The explanation of this difference between

But it was not until 19__ that enough evidence had accumulated to show that the anti-rickets vitamin could not be identical with vitamin A proper—in other words that what had been called vitamin A consisted in reality of two factors, vitamin A itself and *vitamin D the new anti-rickets factor* We have already described how the differences between A and D were detected (p 32) and need not repeat ourselves here

HOW THE IDENTITY OF VITAMIN D WAS TRACKED DOWN

It forms a fascinating story to follow how the identification of vitamin D was eventually arrived at It came about in quite an indirect way namely from a study of the rickets-preventing action of ultra-violet rays

You remember that in 1890 Palm had shown that the prevalence of rickets in different parts of the world had to do with the lack of sunlight. To put it simply sunlight prevented rickets In 1919 it so happened that rickets in a very severe form was raging in Berlin, as a result of the war and the post war blockade. The German Jewish physician, Huldschinsky had the idea of trying to heal the rickets not by natural sunlight which was not always available, but by artificial, ultra-violet, rays instead ('actino-therapy) The experiment was successful and the rickets healed splendidly under the new treatment. At Vienna, too conclusive findings were being obtained by Miss Harriette Chick and her colleagues sent out by the Lister Institute and Medical Research Council. There was no mistaking the prompt healing of the rickets brought about either by antirachitic food (cod-liver oil) on the one hand or by sunlight or ultra-violet rays on the other

The next development, a surprising one, was when it was shown that rickets could be prevented just as well by irradiating *the food* which the animal ate as by irradiating the animal itself This discovery (which was made independently in 1924 by two Americans—Hess a physician and Steenbock a biochemist) was rather disturbing and seemed almost to suggest that rickets could be prevented by some mysterious form of bottled light or energy

dogs and rats will be given later in this chapter and we shall discuss also why certain factors in addition to vitamin D can conduce to rickets or increase its severity while others again alleviate it.

rather than by a concrete substance or vitamin (Indeed some experiments even seemed to indicate that if you irradiated the very *cage* in which the rat lived you could prevent his getting rickets. The true explanation of this strange effect, found later was that there had been some sawdust in the cage



Fig 58 Ultra-violet radiation as cure for rickets.

when it was irradiated and that the rat had later eaten some of the sawdust, which like any other foodstuff had naturally acquired anti-rickets properties from its irradiation!)

But the matter was not to be left in this unsatisfactory position. Science can never progress by advancing semi-mystical ideas about vital energy or vitalism or the like in fact, such philosophies have invariably retarded the progress of knowledge The problem had therefore to be faced in a matter-of

fact spirit—in a materialist attitude if you prefer to call it so The most probable explanation then seemed to be (and it has since proved to be the right one) that under the influence of the ultra-violet light the *vitamin was manufactured*—in the one case in the food exposed to the light or in the other in the body of the animal exposed direct to the light

THE SOLUTION OF THE PUZZLE

We are now it will be seen at last in a position to understand the explanation of the early observations that rickets could be prevented in two apparently quite different ways (1) by sunny climates and (2) by certain foodstuffs such as cod-liver oil. In the first case the vitamin is made direct in your body by the light, in the second you get it from your food.

But now for the final step—to trace the constituent in the food from which the vitamin was formed by the irradiation This obviously was the next clue to take up Accordingly the various components of which food is known to be made up were irradiated separately in turn, to see which would become active It was not long before it was ascertained that it was from the *sterols*, a kind of waxy material associated with the fats in the food, that the vitamin arose

IDENTIFYING THE PRO VITAMIN

Now the principal and best known of the sterols is the one called cholesterol. This, it was found, became intensely active in anti-rickets power when irradiated and for some time therefore it was supposed that the anti-rickets vitamin was the same as irradiated cholesterol In other words, cholesterol was the pro-vitamin being changed to the vitamin by irradiation with the ultra violet rays But later it was found that the true pro-vitamin could not be cholesterol itself but must be some *impurity* present in ordinary specimens of cholesterol—for cholesterol itself it was proved could be got in a highly purified form which could no longer be made active This was shown in turn, in 1926 by Rosenheim & Webster at London Heilbron and his colleagues at Liverpool, and Pohl and others at Göttingen.

The next thing then clearly would be to try and discover the nature of

this impurity present in the cholesterol, and if possible to isolate it. This promised to prove a very laborious and lengthy business but a more subtle way of solving the problem was thought of Why not just try irradiating separately in turn, all the various sterols already known one or other of which was most likely to be the impurity which was being looked for? This would give a simple short cut to the desired destination. So the various specimen bottles were taken down from their dusty shelves and the different sterols were irradiated and tested on rats for anti rickets action Eventually one of them called ergosterol turned out to be the parent substance of vitamin D since it was the one which acquired intense anti rickets properties after irradiation This was found simultaneously by Rosenheim & Webster working for the Medical Research Council at London and by Windaus in Germany

Ergosterol itself had been first isolated many years previously in 1889 by a French chemist, Tanret He had called it ergo-sterol because he had got it from ergot the fungus which grows on rye.

SEVERAL FORMS OF VITAMIN D

For a time it was thought that ergosterol was specific in its properties as pro-vitamin that is to say that it alone and no other substance would acquire anti-rachitic properties on irradiation It has since been found however that there are several other substances which chemically are very closely related indeed to ergosterol, and which will also serve as pro-vitamins Of these perhaps the only one of any consequence is that known as *7-dehydrocholesterol* (or better called *cholestadien-3-ol*) for it now appears that the bulk of the vitamin present in most fish-liver oils is identical with the irradiation product of this substance rather than that of ergosterol.

VITAMINS D_2 AND D_3

Somewhat illogically the vitamin formed from ergosterol has been called vitamin D_2 and that from *7-dehydrocholesterol* and found in most fish-liver oils vitamin D_3 (The substance first named vitamin D_1 turned out to be not a pure vitamin at all but a molecular compound)

Since most plants contain traces of ergosterol, we can assume that the form of vitamin D present in their leaves after exposure to sunlight is D_2 and not D_3

As this edition is passing through the press (1953–4) two new chemical names, *ergocalciferol* and *cholecalciferol* have just been accepted by international agreement, for these two different forms of vitamin D that is for vitamins D_2 and D_3, respectively

For the sake of completeness we have listed in Table LII several other varieties of vitamin D which the research chemists have since succeeded in identifying or making artificially As already hinted these are, however little more than chemical curiosities, and need detain us no longer We have mentioned that they are all remarkably like one another and only differ in some slight detail of structure. This will be seen later in Table LIII.

TABLE LII. *Different forms of vitamin D*

The two important forms
VITAMIN D_2, or *ergocalciferol*
VITAMIN D_3, or *cholecalciferol*
Additional forms (less important)
VITAMIN D_4 or *irradiated 22-dihydro-ergosterol*
VITAMIN D_5 or *irradiated 7-dehydro-sitosterol*
Etc.

For some reason the natural D_3 of fish-liver oils proves much more effective than the synthetic D_2 for preventing rickets—so far as *chickens* or other *birds* are concerned. For *rats* and *human* babies, however the one is as good as the other

ISOLATING THE PURE VITAMIN

But to return, for the time being from these later developments, the next step was to isolate the pure vitamin (D_2 as we should now call it) itself from the irradiated ergosterol. This was harder than might have been imagined and was not to be accomplished for another six years The difficulty is that when ergosterol is irradiated a whole series of very similar substances

are formed alongside one another These are extraordinarily alike in their nature and extremely difficult to distinguish or separate from each other All have the same formula when analysed ($C_{28}H_{44}O$) and an all but identical structure in chemical language they are the numerous isomeric modifications of ergosterol and they tantalize the chemist by forming compounds with each

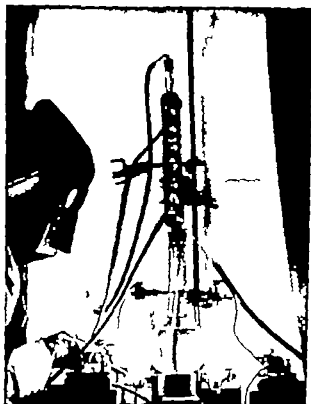


Fig 59 Manufacturing pure vitamin D synthetically *by ultra-violet irradiation.*

other (*molecular compounds*) and generally getting mixed up together yet only one of them is the pure vitamin all the others are inactive.

Eventually however in 193_ after many mishaps and many false hopes, the pure crystalline vitamin D ('D_2) was at last isolated from the mixture. A point of interest is that the task was accomplished simultaneously in the same two laboratories in which the identity of ergosterol as the pro-vitamin had first been established the names of the scientists concerned being



Fig 60 Synthetic vitamin D the finished product with a micro-photograph showing its crystalline form.

Dr R. B Bourdillon and his colleagues at London and Professor Windaus and others at Göttingen.

To come down to the technical, chemical details as to how the separation was effected, it may be added that the German workers used fractional crystallization after treatment with *maleic* and *citraconic anhydride* while the English workers were able to separate the vitamin by converting it into its 3 5-*dinitroben oate ester* The English team proposed the name calciferol

for the pure vitamin-D crystals to remind us that it helps us to calcify our bones properly and that chemically speaking it comes in the category of a sterol

PURE VITAMIN D IN CRYSTALS

The astonishing potency of pure vitamin D and the infinitesimally small quantity of it present in foods may be judged from the fact that it is no less than 400 000 times more active than cod-liver oil. In other words a table spoonful of cod-liver oil (a suitable daily dose to give to a child) contains less than one-millionth of an ounce of the pure vitamin itself, the remaining *399 999* out of the *400 000* parts *of the oil being but the inactive carrier or* filling One ounce of the vitamin is therefore enough to provide a daily dose for over 1 000 000 children!

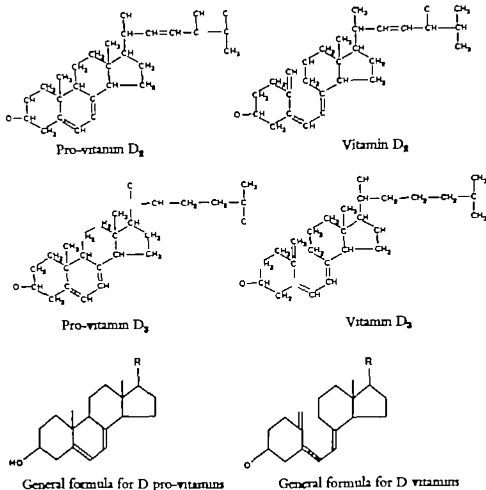
The structure of ergosterol, showing how the atoms are arranged in its molecule, is given in Table LIII the vitamin (ergocalciferol D_2) obtained by irradiating it, is identical with this except for the apparently trivial, but in fact very important and characteristic, change of *a displacement in a double bond and a consequent opening of the ring structure* This too is shown in Table LIII, which further indicates that it is this identical change which occurs also when vitamin D_3 is formed from *its* precursor All forms of vitamin D have exactly the same peculiar ring structure, with only minor and unimportant, differences in the side-chains of the molecule.

VITAMIN DOSES

It was a great boon to the physician to have this *irradiated ergosterol*, or synthetic vitamin D for use in curing and preventing rickets. Sometimes with the old-fashioned remedy cod-liver oil, it was impracticable to give the baby quite a big enough dose to effect a really rapid cure—or to ensure *complete* protection when it was given as a preventive against rickets—especially for example, with negro babies with twins and with premature babies, all of these being liable to develop particularly severe rickets

The advantages of the new remedy then are first that the exact dose can be measured more accurately than with cod-liver oil, which may vary in

TABLE LIII *D vitamins and pro-vitamins*



Pro-vitamin D_2 — Vitamin D_2

Pro-vitamin D_3 — Vitamin D_3

General formula for D pro-vitamins — General formula for D vitamins

potency from one specimen to another and secondly that it is always possible to reach a sufficiently high level of dosage In the words of *The Lancet* calciferol is a remedy that is invariably successful

It is not always realized that to give some vitamin D is not enough you must give a definite optimal amount and no less In other words the vitamin dosage should always be accurately measured out and not just slopped out If you give too little of the vitamin preparation, rickets will

not be entirely prevented. On the other hand it has been found that if you give too much there may be definite harmful effects (p 190) As I pointed out elsewhere (Address to British Medical Association Annual Meeting, 1933) the minimum toxic overdose does not appear to be many times *greater than the optimum curative dose (see Table LIV)* Once again, then, let us repeat adhere strictly and carefully to the dose recommended on the bottle.

TABLE LIV *Vitamin D doses*

	International units per day
Preventive dose	500–1 500
Curative dose	*1,000–3,000*
Toxic overdose	Over 10,000

The medical men who have had the most experience of this particular problem are agreed that synthetic vitamin D (calciferol, now sold under various trade-names) is the most useful remedy for *curing* rickets

For *preventing* rickets a convenient plan, and one which saves much trouble, is to feed the baby on a dried milk preparation in which the right amount of vitamin D has already been *incorporated by the makers* This saves one the trouble of measuring out the fish-liver oil, or vitamin D preparation separately every day Among the various alternatives which have been used are cod-liver oil, halibut-liver oil (more active than the former see p 165) irradiated milk yeast-milk and ultra-violet baths For those professionally interested in the relative merits of these different procedures, a review by the author in the *British Medical Journal* 5 August 1933 may be consulted.

MEASURING THE VITAMIN-D ACTIVITY OF FOODS

To ascertain whether a given sample of cod-liver oil is up to standard activity (which is a very important thing to be able to know) tests are done on rats. The principle is to administer given measured doses of the material to a set of rats suffering from rickets and then find the degree of healing brought about in their bones The more active the preparation the more extensive will

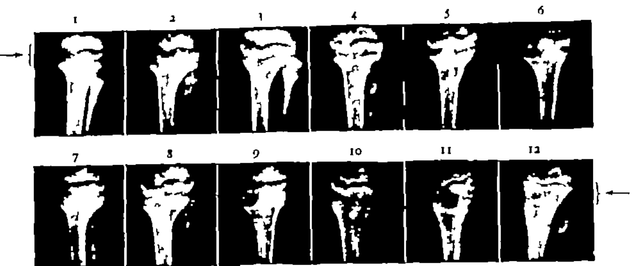


Fig. 61 Rat tests to measure vitamin-D potency of foods.

X-ray photograph of knee-joints of rats with rickets. Varying degrees of healing are shown, ranging from no healing in (1) to maximum healing in (12) (after Bourdillon *et al.*) Compare the appearances of the bone at the position indicated by the arrow the width of the uncalcified gap diminishing as we proceed from (1) to (12).

The degree of healing depends on the amount of vitamin given in the food, and in this way foodstuffs may be measured for their vitamin-D content

be the healing for a given dose (Fig 61) The exact extent of the healing can be measured by X-ray examination or chemical analysis or in other ways, and is compared with the amount of healing produced by a standard material. As with other vitamins (see pp 59 226 etc.) there is an international standard for vitamin D specimens of which are available to persons carrying out tests It consists of a solution of ergocalciferol or vitamin D_2 dissolved in oil. (A later development was the replacement of this by a new standard, consisting of the other form of the vitamin—namely, vitamin D_3. There has also been issued for reference purposes in assay tests, a specimen of cod-liver oil of carefully measured potency)

Sometimes the test is done on young chicks instead of on baby rats. With insufficient vitamin D they develop a special kind of leg weakness as the poultry experts call it

WHICH FOODS CONTAIN VITAMIN D?

Vitamin D stands out from the other vitamins fairly sharply in the fact that *only very few foods* contain appreciable amounts of it. Cod-liver oil and other *fish*-liver oils (especially tunny-liver oil and halibut-liver oil) are extremely rich sources while egg-yolk is relatively good but most other fats (such as vegetable oils and mammalian animal-fats) and milk have but slight activity Other common foods contain the vitamin only in mere traces. Naturally it surprises most people to learn that milk is not highly endowed with the anti-rickets vitamin because they have become accustomed to think of milk as Nature s Complete Food But actual hard experience agrees with bio-chemistry in showing that in our sunless climate it is unsafe to rely on milk alone to prevent rickets, and extra vitamin D must therefore be given artificially to bottle-fed babies.

During the Second World War the British Government saw to it that we all had extra vitamin D by putting it in our margarine, which was fortified with both vitamins A and D Also supplies of cheap or free cod-liver oil, reinforced with vitamin D were made available for pregnant and nursing mothers, and for children (A social survey during the war however

revealed the disconcerting fact that only 40 per cent of the mothers troubled to draw the cod-liver oil to which they were entitled.)

Vitamin D in foodstuffs resists heat fairly well In this respect it stands in contrast with vitamins C or A But it may disappear slowly from foods that have been stored for a long time.

HOW VITAMIN D WORKS IN THE BODY

The picture (Fig 62) is meant to represent in a very diagrammatic way how as I believe, vitamin D functions in the body and prevents rickets. You recall that in rickets an insufficiency of the mineral elements phosphorus and calcium (lime) is deposited in the bones. The diagram indicates that the reason for this is because in rickets (i.e. when the vitamin D is short) the blood fails to bring to the bones enough of the phosphorus[1] or calcium. In fact the function of vitamin D is to raise the level of these mineral elements P × Ca in the blood, which it does by helping you to *absorb and utilize them better from your food*

Thus the effect of vitamin D is to lower the amount of P × Ca excreted from the alimentary tract and so lost to the body hence to raise the amount in the blood and hence to bring enough to the bones to enable them to calcify properly

TABLE LV

The excessive loss of lime and phosphorus in rickets and its effect on the bones

	Loss of Ca in faeces (expressed as per centage of intake)	Loss of P in faeces (expressed as per centage of intake)	Composition of bones (percentage of ash in bones)
Normal	20–40	15	55–60
Rickets	90–100	60–70	20–30

The accompanying table (Table LV) and Table LVII (p 192) illustrate these points statistically and show that it is a fact that in rickets you do have too

[1] More strictly *inorganic phosphate* The other forms of phosphate present in the blood seem little affected by a deficiency of vitamin D This applies to all the following discussion, the symbol P being used for simplicity

much P and Ca lost by excretion in the faeces too little circulating in the blood, and too little laid down in the bones

Now a good deal of evidence can be cited in support of the above theory Thus it agrees with the observation that, when measures are taken to cure the

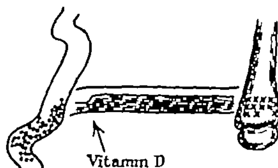


Fig 62 How vitamin D works

With more vitamin D there is increased net absorption of Ca and/or P from the intestine, their level in the blood rises, and so more is precipitated in the bone.

By an increase in the net absorption is meant either that more is absorbed through the wall of the intestine into the blood stream or alternatively that less is re-excreted back again from the body into the intestine. The fact that less is lost in the faeces does not in itself enable us to decide which of these two balancing actions is most involved.

Although the net absorption is increased by vitamin D we have no evidence that the vitamin is necessarily acting directly on the absorptive functions of the gut as such. All that we can say is that vitamin D enables more P and Ca to be carried into the blood—much as though it were increasing their solubility in the blood. The extra P and Ca will be drawn from the intestine if they are available there. Under exceptional circumstances they may be taken from the mineral stores present in the shaft of the bones.

The latest evidence seems to suggest that an initial effect of vitamin D in the body may be to cause an increased turn-over in the soft tissues, of phosphate from its combined, or *organic* form to that of *inorganic* phosphate. The other changes described above and in the text—the rise in the level of P in the blood, consequent increased calcification, etc.—may possibly therefore be secondary to that primary action.

rickets the first change noted is that the low mineral content of the blood is put right, and then the healing of the bone follows after this That is the bone cannot start being calcified properly until a sufficient concentration of building-stone material is brought to it by the blood. This agrees very

nicely with experiments which can be done in test-tubes in which it i[s shown] that the amount of healing (re-calcification) taking place in a piece of [rachitic] bone kept in blood or other similar solution varies with the amoun[t of P] and Ca in the solution Such experiments were carried out by Ship[ley in] America and by Robison in England

VITAMIN-D ACTION—SOME TECHNICAL CONSIDERATIONS

A great many other facts might be cited in agreement with the th[eory] advanced above but some are perhaps rather technical. Such are that w[hen] we give a rat his P in a relatively insoluble form (which can be done [by] various means) it makes his rickets worse—clearly because it is harder for [an] animal to absorb any material which is less soluble Alkaline food has [the] same effect, and so similarly increases the severity of rickets

In a similar way rickets can be intensified by the addition of overmuc[h] cereal food to the ration The reason for this is that the cereals contain muc[h] of their phosphate in a particularly insoluble form, called phytic acid. Th[is] phytic acid-P is not only insoluble itself (and hence rickets-provoking) bu[t] also to make matters worse it forms insoluble salts with the calcium in the food.[1]

Again if we give the rat a diet very short of P but with plenty of Ca we then find (as may be expected from the theory) that it is the P which tends to be short in the blood and that the Ca is normal. The animal develops what is called low P rickets. Under the reverse conditions he develops low Ca rickets there being relative deficiency of Ca available in the food it is in this element that his blood becomes impoverished

[1] *A footnote on the national loaf* There happens to be more of this rickets-promoting phytic acid in whole-grain wheatmeal flour than in white flour It was argued that this fact constituted a possible objection to the use of the new national wheatmeal flour—and the national loaf—introduced into Britain during the Second World War With this criticism in mind, the Government in the United Kingdom took the precaution of adding calcium carbonate (a variety of purified chalk) to the national wheatmeal loaf—with the aim of rectifying any such relative deficiency of calcium. Not too serious view need be taken, however of this phytic acid in our flour because it has been shown that in the baking of the bread—or rather during the raising of the dough—much of the phytic acid is converted to a more assimilable form in technical language the insoluble inositol-phosphoric-acid is hydrolysed into assimilable phosphate

The theory helps us to explain too why rickets develops less readily in rats than in dogs (p 174) I found that rats (even normal rats) are able to absorb any trace of P and Ca from their food much *more easily than are dogs*. Therefore it is clear you must take sterner measures with the rat than the dog if you want to increase his excretion of P and Ca and so give him rickets. This you have to do by upsetting his mineral ration, making it relatively insoluble and thus difficult to absorb and the rat duly gets rickets.

But surprisingly enough, some of the principal evidence for this theory came from a study not of vitamin lack (*a-vitaminosis*) or of partial shortage (*hypo-vitaminosis*) but of vitamin excess (*hyper-vitaminosis*) The ill effects which could be produced by giving overdoses of vitamins formed a new and interesting branch for research.

HYPERVITAMINOSIS

The fact that very large doses of irradiated ergosterol might be harmful to animals was first noticed by Pfannenstiel and Kreitmair & Moll in Germany and was before long to be confirmed by many others. But they did not understand the nature of the condition or examine the blood calcium and phosphorus or the *chemical metabolism* and it was supposed by many that the ill effects were due merely to some toxic by-products and not to vitamin D itself at all, so novel did the idea then seem that a vitamin could be anything but beneficial!

At this stage I predicted, from the above theory of the mode of action of vitamin D what the effects of overdosage ought to be, and then when we

TABLE LVI. *Results of vitamin excess (hypervitaminosis) and vitamin deficiency (hypovitaminosis) compared*

Deficient vitamin D	Normal vitamin D	Excessive vitamin D
Deficient absorption of Ca and P " Ca and P in blood calcification	**Normal** absorption of Ca and P Ca and P in blood calcification	**Excess** absorption of Ca and P Ca and P in blood calcification

came to investigate the actual symptoms they turned out to be indeed as we had expected

I argued that just as lack of vitamin D caused shortage of the mineral elements (Ca and P) in the blood and a resulting insufficient mineralization

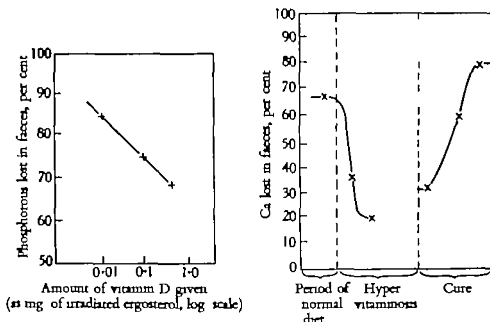


Fig 63 The effect of vitamin D in diminishing the body's loss of lime and phosphorus.

Left as more vitamin D is given in the diet, the loss of phosphorus in the faeces is decreased. Right the loss of calcium followed graphically during the course of an experiment with excess of vitamin D Where the excess is first given the amount of calcium excreted in the faeces immediately falls. As the animal becomes severely ill secondary effects associated with starvation are seen (dotted line). Finally when the excess of vitamin D is removed from the diet an increased amount of Ca is again lost in the faeces.

(Dotted line = result of toxic effects with maximum overdoses.)

of newly forming bone and again as moderate amounts of vitamin brought these up to normal values, so therefore with *excess* of the vitamin one should expect to have *excessive* Ca × P in the blood and so *over-calcification* in the newly forming bone—that is if the theory were correct. Experiments were accordingly undertaken and analyses carried out to test whether these effects

TABLE LVII *Bad blood as the cause of rickets The blood in rickets and hypervitaminosis*

	Rickets (no vitamin D)	Normal (with vitamin D)	Hyper-vitaminosis (excess vitamin D)
Amount of phosphorus (mg per 100 ml. of blood)	3	4	8
Amount of calcium (mg per 100 ml of blood)	7	10	15

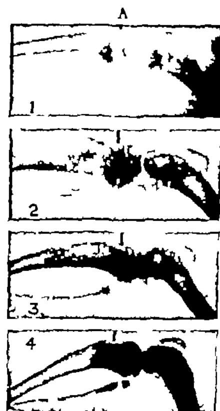


Fig 64. Vitamin-D deficiency and excess comparison of effects on bones.

X-ray photograph of knee-joint of rats 1 Rickets—*insufficient* calcification at growing end of bone. 2, Normal—*normal* calcification at growing end of bone. 3 4. Hypervitaminosis—*excessive* calcification at growing end of bone.

(Compare effects at positions marked, under A.)

did in fact appear and it was satisfactory to find that they did (see Table LVII and Figs 63–66) The amount of calcium and phosphorus in the blood was sometimes almost double the ordinary normal figure in fact it was so grossly overloaded or supersaturated that it was not surprising to find that the excess of minerals seemed to be simply precipitated, shot out of the blood wherever there was the opportunity *In consequence hypervitaminosis caused*



Fig 65 Ill effects of excessive doses of vitamin D on the teeth. Enlarged section showing new calcium deposition around teeth (C_3) causing rigid attachment to jawbone.

some remarkable abnormalities such as a deposition of calcium salts in and a consequent hardening of, the arteries the formation of kidney stones and stony deposits in other parts of the body The new Medusa! Various other symptoms, too were seen which could be accounted for on theoretical grounds e.g the excess of calcium in the blood resulted in the kidney excreting it in large amounts and the urine became thick and cloudy with it. The teeth were excessively mineralized on the surface and in fact sometimes joined on rigidly to the jawbone by the calcium deposits (the so-called *ankylosis*) (see Fig 65)





66 Ill effects of excessive doses of vitamin D on the kidney (above) and aorta (below), the black-staining areas representing hard calcareous deposits.

HYPERVITAMINOSIS IN BABIES

About this time there were some instances, in America, England Germany and elsewhere of babies being given not enough vitamin D but too much Ill effects resulted and it was noted that these were identical with those which we had first seen in our experimental animals—i e excess of the mineral elements in the blood, loss of calcium by way of urine increased density in newly forming bone with symptoms of fever etc.

This trouble is less likely to happen now—now that the danger is understood. It is probable that at least one death unfortunately occurred at the time as the result of hypervitaminosis but such an occurrence need not be feared again Should any indisposition be noted as a result of excessive administration of the vitamin once the dosage is reduced to a more reasonable level the baby very soon becomes well again and suffers no permanent harm. It would be a pity if fears of its possible abuses led to any hesitancy in the use of so valuable a remedy (p 165)

VITAMIN D AND THE PARATHYROID GLAND

A number of investigators noted that the effects of vitamin D in large doses seem to resemble those of the natural secretion or hormone, elaborated by the parathyroid gland Both, it was known had the effect of increasing the amount of calcium in the blood The theory was therefore advanced, naturally enough, that perhaps vitamin D acted by stimulating the parathyroid secretion

Experiments carried out in my own and other laboratories however proved that vitamin D worked quite differently from the parathyroid—in fact in a sense in opposition to it The difference is this. Vitamin D helps us to keep sufficient calcium in our blood by improving its assimilation from the food—that is by diminishing the loss in the faeces. The parathyroid on the contrary increases the calcium in the blood *by drawing it out of the bones* So far from helping us to assimilate the mineral elements from our food, it *removes* the calcium out of its stores in the body

Now we know that the explanation of convulsions in babies due to nutritional tetany is that they have too little calcium in their blood. Treatment with parathyroid extract had accordingly been advocated for this and also for certain other supposedly similar conditions From what is now known this would certainly seem a mistake, in any way except as an immediate emergency measure. For while treatment with the parathyroid extract undoubtedly helped to overcome the *symptom* it only aggravated the underlying error The real cause of the convulsions, after all, was inadequate utilization of the calcium (lime) of the diet too much was being lost by excretion Treatment with parathyroid only made this worse, drawing calcium into the blood at the expense of the bones and ultimately still further impoverishing the already starved body The correct treatment, generally for a low blood calcium is administration of vitamin D together of course with adequate provision of calcium itself. Such treatment, as we see, will result in less of this element being lost by excretion, and so in its better retention and utilization by the body

VITAMIN D IN SURGICAL TETANY

In another direction, vitamin D has a use in relation to tetany *In adults, it may occasionally happen that because of a malignant growth in one of the parathyroid glands some degree of surgical extirpation becomes a necessity In consequence of the surgical damage then done to the glands, there may* follow some temporary loss of the parathyroid secretion and hence a precipitate fall in the level of calcium in the blood, which in turn causes severe attacks of tetany To control this hazard it has now become the recognized procedure to administer large doses of vitamin D immediately after these operations.

This practical application of vitamin D in cases where no deficiency as such exists, may be described as a *pharmacological use* This is to distinguish it from its more usual use, in repairing a deficiency as in the cure or prevention of rickets (or of infantile tetany) which, by contrast, can be described as *replacement therapy*

VITAMIN D IN LUPUS

Another and even more remarkable pharmacological use of vitamin D is for the cure of lupus—or to give the disease its correct name, lupus vulgaris a tuberculous affection of the skin causing severe ulceration and disfiguration

In the middle 1940's it was discovered that lupus could be cured in the most spectacular manner by the administration of massive doses of vitamin D The amount of the vitamin needed for the purpose is very high indeed—sufficient to provoke some mild degree of hypervitaminosis (see pp. 190–5) Hence, very careful control of the dosing by experts, is necessary It is probable that the vitamin D acts by promoting local calcification of the tuberculous lesions within the skin and hence in their consequent rapid healing

VITAMIN D AND DENTAL DECAY

One final word about vitamin D must be added here. Just as an adequate supply of vitamin D helps to build sound, healthy bones, so it is similarly needed for the formation of good hard, normal teeth. Probably well-formed teeth are less likely to become decayed than are teeth of inferior architecture. Hence, much has been written about the probable value of vitamin D in preventing or even curing dental disorders However vitamin D is not the sole dietary factor concerned in the production of normal teeth and probably not the most important one. Hence it is probably best to defer our discussion of these problems to a later stage in this book (p 332)

CHAPTER VII

VITAMIN A

The photographs below (Fig 67) illustrate a peculiar kind of eye dise marked by inflammation and infection and known to the oculist as x ophthalmia. These photographs were taken by Prof. C E Bloch at Cop hagen in 1917 at a time when dozens of cases were occurring there amo



Fig 67 Xerophthalmia—an eye trouble caused by deficiency of vitamin A. The cause thousands of cases of blindness annually in India.

babies as a result of war conditions Milk products were being exported t Germany with the result that the Danish babies went short But this same ey trouble had been known for long years previously David Livingstone, th famous explorer in his book published in 1853 refers to its prevalence amon both man and beast in the Dark Continent Already in 1848 a *Treatise o Cod-liver Oil* published in Edinburgh tells how this eye disease may be cure with cod liver oil and in 1904 an investigator in Japan, Mori, gave an elaborat

account of large numbers of cases occurring among under nourished children and relates how he had used cod liver oil successfully for its cure



Fig 68 David Livingstone (1813–73), the explorer who referred to the prevalence of xerophthalmia in Africa. He is here seen in a typically Victorian period photograph, with his youngest daughter Anna Mary

At the time we are speaking of xerophthalmia was not yet recognized as a vitamin-deficiency disease But numerous scientists had made experiments by feeding animals on purified diets and had noted that xerophthalmia resulted

from the absence of good fats and when fat-soluble A came to be distinguished from water-soluble B in 1915 it was almost immediately realized that it was the former (the fat-soluble A) which was specially concerned in preventing this trouble, and not the water-soluble B

DRY EYE

Now fat-soluble A or vitamin A was usually tested for by experiments on the growth-rate of rats so that vitamin A was sometimes known as the growth-promoting vitamin But this name is rather a misnomer because, after all not only vitamin A but *all* the vitamins (as in fact all dietary essentials whether minerals, protein, vitamins or what not) are obviously necessary for proper health and therefore for full growth. Vitamin A if a label is needed would better be described as the *anti-xerotic* vitamin That is to say it prevents this *xer*-ophthalmia and various other rather similar troubles throughout the body which likewise seem to have their origin in the *xerosis*, or kind of dried-up condition of the membranes of the body which occurs in its absence. *Xer-ophthalmia* translated into English means simply dry eye

The first change which can be detected in xerophthalmia is in the *conjunctiva* the delicate membrane surrounding the eye, which becomes dried, thickened and crinkled Sometimes in cases of prolonged mild deficiencies of vitamin A a careful examination will reveal certain characteristic, chalky looking white spots named Bitot s spots after C Bitot, a physician at the foundling hospital in Bordeaux, who first described them as long ago as 1863 In severe cases the state of the eye is likely to deteriorate rapidly the cornea—the horny covering of the eye-ball—may become opaque, weakened, and necrotic (a condition described as *keratomalacia*) sight is lost, and before long ulceration may cause destruction of the eye. If however vitamin A is given in time the eye will be saved.

VITAMIN-A DEFICIENCY IN THE WORLD TO-DAY

Any *severe* deficiency of vitamin A, causing advanced symptoms of xerophthalmia, is relatively rare in this country But it is not quite unknown for example, seventeen cases of xerophthalmia were seen in the course of a year





Fig 69. Xerophthalmia in dogs and rats a condition preventable by animal fats (e.g. milk fat, cod-liver oil, etc.) or other sources of vitamin-A activity

in one hospital alone in the north of England. On the other hand, *mild* degr
of vitamin-A deficiency have probably been fairly common. For examp
my colleagues and I found, a few years before the Second World War th
one elementary school child out of every three in certain poor slum areas
the East End of London, appeared to have a slight degree of deficiency
vitamin A as detected by a test for partial night-blindness to be describ
below on p 207 What confirmed our diagnosis was an independent repo
by the Chief Medical Officer for the L C C published later his inquir
showed that those children whom we found to be affected were not receivi
milk in school and generally had little or no fresh milk even at home (som
times only a little condensed) At the present time with the great grow
of the milk in schools scheme the compulsory vitaminization of margarin
and the provision of cod-liver oil or vitamin concentrates for mothers an
babies, things have undoubtedly greatly improved, and any vitamin-
deficiency among school children must now be rare.

But the position is far more grave in many other parts of the world. I hav
already alluded to the outbreaks in Denmark in the First World War, an
the numerous cases in Japan To this day in India it is still *the chief cause*
preventable blindness in children according to Professor R. E. Wright and oth
ophthalmological authorities One recent medical writer refers to sixty-seve
cases in one year seen in Madras two-thirds of these children become pe
manently blind. Equally distressing accounts could be given from parts o
China, from Yucatan and from Java and Sumatra and the Malay States.

NIGHT-BLINDNESS

It is in children especially that lack of vitamin A causes this eye infection
xerophthalmia. In grown-ups however the eye does not generally becom
actually infected long before this another symptom is noted, and a ver
interesting one too namely *night-blindness.* This means simply as its nam
implies inability to see in a dim light. Night-blindness in a really acute form
has been quite common in parts of Newfoundland and in Labrador in China
Brazil, the Dutch East Indies and elsewhere

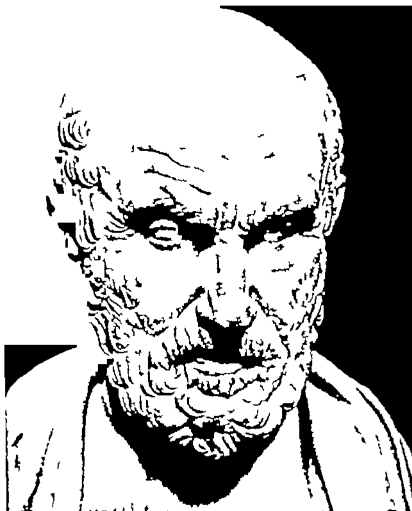


Fig. 70. Hippocrates (ca. 500 B.C.) who knew of night-blindness (a form of vitamin-A deficiency) and its cure by liver

It was alluded to of old by Hippocrates who even adds the very accu modern note that ox-liver cured it He was quite right!

Rats among other animals, also develop *night-blindness when depr* of vitamin A—as an ingenious observer has proved by putting them c revolving turn-table and watching how they jump off in a darkened ro Similarly calves fed on inadequate diets have been noticed to run into brick walls of their pens instead of through the gate

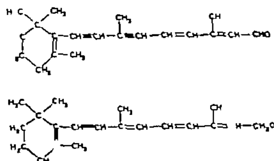
Very interesting is the explanation of this connexion between night vi and vitamin A One s ability to see depends, among other things, on presence of a peculiar colouring matter called the visual purple foun the retina, at the back of the eye. The retina is to the eye much what photographic plate or film is to the camera and the visual purple may compared with the sensitizing agent in the photographic emulsion. T *visual purple gradually gets used up particularly when the eye is expose* a bright light and in consequence needs to be regenerated. Now it is fo that in vitamin-A deficiency there is insufficiency of this purple matter a this it is which has to do with the difficulty of seeing in the dark G sufficient vitamin A the visual purple reappears and you can see norma again—within a matter of hours Clearly then vitamin A is needed for t regeneration of the visual purple.

A fairly full chemical explanation can be set out. Visual purple is a comp substance containing vitamin A bound to protein, and unless the blood is a position to furnish a regular supply of the vitamin to the retina the mar facture of fresh visual purple is held up The chemistry of the transaction *noted in Table LVIII and Fig 71 (opposite)*

NIGHT VISION AS TEST FOR VITAMIN-A STATUS

The earliest detectable result of any shortage of vitamin A *from the diet* th is some degree of night-blindness however slight Hence the most delica method we know for telling whether a person is in an optimum state nutrition in vitamin A is by tests on his eye A measurement is made of wh is called **dark-adaptation.** What this means can be explained as follows.

TABLE LVIII *Retinene or vitamin A aldehyde (above) a substance occurring naturally in the retina of the eye and concerned in vision It is closely related chemically to vitamin A itself (below)*



A few years ago we were all of us familiar with the fact that a normal individual, on coming out of a brilliantly lit room into the war-time black-out, found that he could not at first see dimly illuminated objects. Little by little his vision improved so that, after a time, objects at first barely perceived in the black-out appeared relatively bright and soon almost brilliant. Then

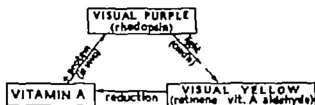


Fig 71 Vitamin A and vision. An outline of the chemical changes occurring in the retina of the eye—the vitamin-A cycle

he came to distinguish, one after another objects more and more faintly illuminated. This process of progressively improving perception eventually slows down until after about half an hour or three-quarters of an hour a final steady state is reached The process is called dark-adaptation. Another familiar example of it is seen every time we leave the bright daylight and enter the cinema.

People short of vitamin A adapt too slowly as compared with well-nourished people, and they will completely fail to see a light so dim as to be just barely perceptible to normal people. This is true, no matter how long they continue to stay in the dark. The deficient subject may himself be entirely unaware of any abnormality as it is not always sufficiently severe

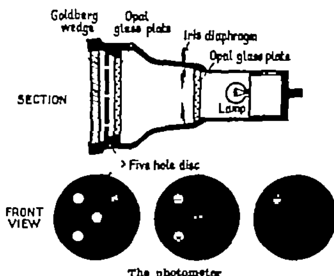


Fig 72. Visual photometer An instrument for measuring visual acuity

In the type of instrument here illustrated, the subject to be tested is instructed to look down a tube, shown in section above. Inside this tube is visible a disc with 5 illuminated holes, arranged in a dice-pattern, and represented in the plan below By means of a device called a Goldberg wedge, the holes towards the right-hand side are maintained very slightly less brightly illuminated than those towards the left.

The operator then slowly diminishes or increases the total degree of illumination of the 5 spots until the subject reports that he can see more than 2 of them, but less than 5 (namely 3). A pointer on a dial then informs the operator what is the exact degree of illumination needed for the 3 spots to be just barely visible in this way

The process is repeated at intervals during a stay in the dark, and thus the complete dark adaptation curve is traced.

to produce any appreciable disability However the worse the degree of deficiency in the vitamin, the poorer the rate of adaptation, and the more severe the degree of night-blindness—and in consequence the brighter the light needed in order for it to be perceived. Hence we have a convenient method of assessment of the *degree* of deficiency The procedure is as follows

The subject to be tested is first told to gaze at a brilliant lamp of standard brightness for say five minutes The light is then switched off and the subject sits in the dark for the rest of the test. At intervals of perhaps every five minutes he is asked to peer into an instrument called a visual photometer (Fig 72) and to say whether he can detect various dimly illuminated spots of light of varying standard grades of intensity On each occasion the dimmest

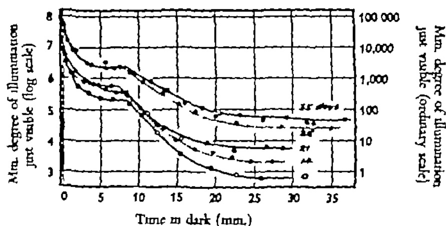


Fig 73 Dark adaptation curves, showing progressive deterioration in dark adaptation after periods of 14, 21 25 and 35 days on a diet devoid of vitamin A.

It will be seen from the bottom curve, that the minimum degree of illumination which is just visible to a normal subject, after staying 10 minutes and 30 minutes in the dark, is of the order of 100 and 1 respectively

But after a period of restriction to a diet deficient in vitamin A for 35 days, the sight had so much deteriorated that a degree of illumination of almost 100 units, instead of 1 unit was needed, after 30 minutes in the dark (read on right-hand scale) or of 1000 instead of 100, after 10 minutes in the dark.

spot just visible to him is noted (There are various ways of checking his veracity such as by asking him the shape of the spot or to say which way an arrow is pointing)

Thus we can trace out a curve showing the subject s gradually improving vision in the dark, during the half-hour or so of the test the so-called dark-adaptation curve (Fig 73) If the diet has contained inadequate vitamin A, the curve will be out of place shifted upward from the dark to the bright

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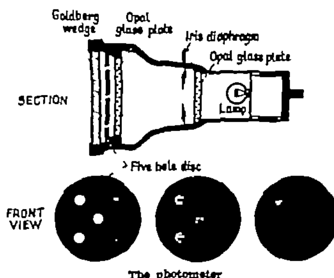


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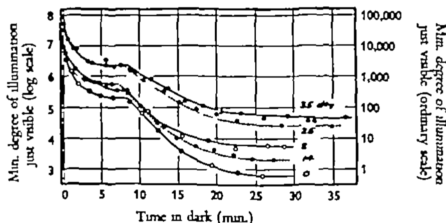


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side of the graph. The worse the deficiency the more displaced the curve. B
in order to satisfy ourselves beyond any possible doubt that the error is in f
due to vitamin deficiency and not possibly to some organic disease of
eye,[1] it is advisable to make a control test. For this purpose the subj
thought to be deficient is given vitamin A in large doses, and his dark adap
tion tested again after a few days If an improvement results, it is a conf
mation that the tentative diagnosis of vitamin deficiency was correct. In o
tests on school children (p 202) we used a double check. Some of the childr
were given no vitamin and it was found that their performance remain
stationary Others were at first given none, and with the same result, b
were later dosed with the vitamin and it was found that an improvement th
followed in most instances.

At various times stout-hearted volunteers have lived on diets deficie
in vitamin A, and watched the gradual deterioration in their dark-adaptati
curves (Fig 73 and see below p 234) By their services they have increas
scientific knowledge, and so helped suffering humanity

THE VARIOUS EFFECTS OF VITAMIN-A DEFICIENCY IN RATS

As we have already said, rats like human beings, deprived of vitamin A soo
cease to grow at full rate and may develop eye trouble (xerophthalmia) an
night-blindness. But there are certain other troubles too which althoug
less obvious are to be seen for the looking

Generally the vitamin-A deficient rat dies with some infectious troubl
in the lung (such as broncho-pneumonia) or in the alimentary canal, o
elsewhere. This susceptibility to infection was noted by McCollum i
America in some of his earliest work on fat-soluble A in 1917 and has bee
repeatedly confirmed by later investigators. Later on in the chapter we sha
discuss also certain other ill effects which may result from lack of vitamin A
including abnormalities in the structure of the gums round about the teeth
and in the kidney (sometimes causing stones in the kidney)

[1] Dark adaptation is also affected in some relatively rare disorders of the eye, e.g. r
retinitis pigmentosa.

But strangely enough the most essential feature of all and in all probability the cause of most of these other troubles was for long generally overlooked It is this that in the absence of vitamin A the mucous membranes, those delicate lining fabrics throughout the body tend to lose their normal structure and cease secreting a proper supply of mucus—they become dried up (p 200) In scientific parlance the cells of which the membranes are constructed undergo a *metaplasia* i e. a definite change in character and appearance. Instead of remaining moist and actively functioning they become dry horny and flaky or keratinized —appearing in fact much more like our ordinary external skin, or cuticle. The principal role of vitamin A then seems to be to keep these cells functioning properly and to prevent their degenerating in this manner This effect on the membrane was first noticed by an observer in Japan Mori in 1922, and later by two Americans, Wolbach & Howe, in 1925 and I emphasized its fundamental significance in a paper in *The Lancet* in 1932.

IS VITAMIN A AN ANTI-INFECTIVE AGENT ?

When the first edition of this book was published, vitamin A was often spoken of as the anti-infective vitamin and it was suggested that if you took enough of it it would prevent your catching coughs and colds and the commoner infectious diseases such as measles chicken pox, or scarlet fever etc. It was even claimed (and widely boosted in the advertisements in the newspapers) that should you have already caught one of these infectious diseases a dose of vitamin A would help you to recover more quickly

How did this theory start? Well, it was known that animals dying from vitamin-A deficiency became infected, and it was assumed rather too easily that a kind of generalized converse was true also *that is that infections are* due to vitamin A deficiency The logic of course was at fault, and the theory also in my opinion at variance with the known facts

THE CAUSE OF THE INFECTIONS IN VITAMIN-A DEFICIENCY

What we have to ask ourselves is *Why* do these animals suffering from vitamin-A deficiency become infected? In an investigation of this question by Dr Innes and myself, the conclusion was reached that the infections which one sees in vitamin-A deficiency are after all of a rather special character and have but little in common with the ordinary infectious diseases prevalent in man in this country The actual cause of the infections in vitamin-A deficiency seems to lie simply in the disordered condition of the mucous membranes. Becoming dried up in the manner described above, and their ducts choked up with the flaky particles of skin being continually cast off from them, they naturally formed a favourable breeding ground for any chance micro-organism which might happen to alight on them for as we know the air our food, and all our surroundings are normally teeming with such microbes which would ordinarily be quite harmless to us

This indeed was what happened. There were many such scattered local infections throughout the body in the vitamin-A deficient rats but they always had their origin in such disordered, keratinized membranes. When the rat was given a supply of vitamin A, the membranes after a time began to function normally again and the infesting microbes were washed off, as it were with the result that the local infections gradually disappeared and the animal became well.

It was pointed out therefore that *these peculiar local infections* had really *little to do with common infectious diseases in man* as ordinarily met with in this country the latter after all being generally due to definite virulent or pathogenic, disease-producing micro-organisms, which gain their entry into the blood stream—a different story indeed from the accumulation of chance, non-virulent microbes on the weakened membrane tissues which is what happens ordinarily in the vitamin-A deficient animal.



Fig. 74. Effect of vitamin-A deficiency on membrane structure

The photograph shows the cell structure magnified 850 times, of the epithelium of the trachea The want of vitamin A has caused the normal epithelium A to A, to be replaced and undermined by a scaly type (*stratified* and keratinizing) *B* to *B* (After Wolbach & Howe)

VITAMIN A DOES NOT INFLUENCE IMMUNITY

Further experiments confirmed an earlier observation showing that when animals were deprived of vitamin A they did not lose their power to form antibodies that is to resist the attack of disease-producing agents entering the blood stream—as might have been expected if vitamin A were indeed a general anti-infective agent as was suggested. In other words vitamin A had no effect on immunological reactions, and was not *in this sense* the anti-infective vitamin increasing the natural resistance to all types of germ diseases.

In further confirmation of this view Dr Griffith and I showed that rats infected with tuberculosis bacilli in their blood, resisted the germs no better or worse whether they had vitamin A in their food or not.

VITAMIN-A RESERVES OF HUMANS

But perhaps one of the most convincing pieces of confirmatory evidence came from my colleague Dr Thomas Moore. He was carrying out post-mortem examinations of the *vitamin-A reserves* of human beings. The vitamin A of our bodies is stored up in the liver so that an analysis of this organ after death for its vitamin-A content will tell you whether the individual had sufficient vitamin-A reserves for the needs of his body or not. Now Dr Moore found that people might die from various common infections with *ample vitamin-A reserves* The conclusion seemed inevitable therefore that it would have been futile to have tried to cure them by giving them extra vitamin A when they already had enough.

LIMITATIONS OF VITAMIN A AS ANTI-INFECTIVE AGENT

At one time great stimulus was given to the theory that vitamin A increased e resistance to septicaemias and infectious diseases generally by some results hich appeared to suggest that blood poisoning in child-birth fever *puerperal epticaemia* a particularly dangerous condition, might clear up when treated

with vitamin A. Later tests, however, indicate that this result was probably largely accidental, as attempts to repeat it have been generally unsuccessful. Furthermore, in subsequent clinical trials, vitamin A has been tried, but without effect, as a preventive for common colds and similar respiratory infections, or in the curative treatment of pneumonia, scarlet fever, tuberculosis, etc.

The position therefore in a nutshell is this. If you take insufficient vitamin A, you are admittedly liable to develop special kinds of localized, low-grade infections. It is wise, therefore, to take care that your diet is adequate and well balanced. But this has nothing to do with ordinary acute infectious diseases as commonly met with, and since, moreover, most people in this country do appear to get adequate amounts of vitamin A, it would seem illogical to expect to cure such infectious diseases and septicaemias with vitamin-A medication.

VITAMIN A AND AN UNHEALTHY SKIN

Now when we speak of the 'common infectious diseases', we have in mind such conditions as measles, scarlet fever, rubella ('German measles'), diphtheria, chicken-pox, typhoid fever, whooping cough, mumps and smallpox, as well as the common cold and influenza. Whenever tests have been carried out with vitamin A with the object of preventing such infectious diseases or of aiding their cure they have nearly always given disappointing results. It would be more reasonable to look for the first effects of vitamin-A deficienc then in an unhealthy condition of the mucous membranes or

A characteristic change in the skin sometimes noted by the vitamin-A deficiency is that it becomes excessively dry and develops a typical *papular eruption*. The dry, horny papules meas from a pin's head to a quarter of an inch in diameter.

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It seems therefore that while an unhealthy condition of the skin may sometimes owe its origin to a lack of adequate vitamin A it is not very often the responsible factor in this country

TOAD-SKIN IN CEYLON

But it must not be assumed that there is no shortage in other parts of the world. In Ceylon for example, Dr L. Nicholls has pointed out that the patients in the asylums, and the children in the poor vernacular schools, do not receive even 30 per cent of their *calculated minimum needs* of vitamin A. In Ceylon xerophthalmia is so common that no less than two-thirds of the numerous cases of blindness in children are said to be due directly to it. But, according to Dr Nicholls, the earlier symptom is an *inflamed and coarsened* condition of the skin to which because of its appearance, he applies the graphical name toad-skin Toad-skin together with a condition frequently associated with it, sore mouth prevails to an astonishingly wide extent among the Sinhalese schools, gaols, asylums and hospitals (see Table LIX) It clears up Nicholls states when vitamin A (e.g as cod-liver oil) is given or when a more nourishing diet is substituted. We now believe that other

TABLE LIX. *Toad-skin in Ceylon*

Subjects	Percentage affected
In charity boarding schools	83
In poor vernacular schools	29
In upper-class schools	3
In mental asylums (native diet)	44
In mental asylums (special European diet)	2

nutritional deficiencies may be involved, as well as that of vitamin A in causing toad skin Nevertheless, we see from this table how much economic conditions may influence nutrition

TEETH GUMS, NERVES AND BONES

Not only the mucous membranes are affected by vitamin A deficiency The cells in other parts of the body also may degenerate in its absence Such structural abnormalities have been described for example in the teeth and in the nervous system.

The bone and tooth cells have been examined particularly by Wolbach & Howe in America. The cells become atrophied in the absence of vitamin A, and normal tooth structure is impossible

The gums, again (like other membrane tissues) suffer structural changes—namely a thickening or hyperplasia—and so become liable to infection and as Lady (May) Mellanby has suggested, a lack of vitamin A in childhood may perhaps constitute a predisposing cause of pyorrhoea in later life.

The effect of vitamin-A deficiency on nerve cells has also been much studied. Professor E B Hart and his co-workers Miller & McCollum in the U S.A seem to have been the first to report (in 1914–16) that animals deprived of fat-soluble A might suffer degeneration of the cells of the nervous system and develop symptoms of lack of co-ordination with a staggering gait, or spasms These observations were made on pigs and it is interesting to call to mind that such ill effects were later observed by us at Cambridge to occur spontaneously on pigs kept on a natural diet recommended as being complete but actually lacking in vitamin A In 1921 Steenbock in America made similar observations, using dogs as his experimental animals and Hughes also in America published in 1928–9 a detailed account of nerve degeneration and symptoms of incoordination and spasms seen in vitamin-A deficient chicks cows, pigs and other animals

More recently the problem was studied intensively by Sir Edward Mellanby already in 1926 he had found that puppies kept on a diet low in fat-soluble A developed nervous irregularities which he attributed to a toxic

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effect of the excessive amounts of cereal in their food. Mellanby asks whether this nerve degeneration may not even govern the degeneration of the membrane tissues and the xerophthalmia itself seen in vitamin-A deficiency Also as rather similar degenerative changes are seen in certain other diseases in man might not vitamin-A deficiency possibly enter into any of these? The answer however appears to be that nerve degeneration of a somewhat similar appearance may arise from a variety of different causes as well as from lack of vitamin A.[1]

Another abnormality seen in an animal deprived of vitamin A, and studied by Mellanby is an overgrowth of bony tissues This may occur for example in the skull. As a result of the bony overgrowth pressing on the optic and auditory nerves there may be interference with vision and hearing

To conclude the list of bodily defects and disorders known to follow upon deficiency of vitamin A is now quite lengthy For the sake of recapitulation and summary we have enumerated them in Table LX.

TABLE LX. *List of disorders resulting from lack of vitamin A in animals*

(1) Night-blindness
(2) Failure of growth (in the young)
(3) Xerophthalmia (dry eye) and consequent keratomalacia (softening or destruction of the cornea of the eye)
(4) Xerosis (drying) and metaplasia (changed structure) of epithelial tissues
(5) Secondary infection, consequent on no 4
(6) Calculi (stones) in the kidney
(7) Hypertrophy (enlargement) of gingivae (gums)
(8) Failure in reproductive function
(9) Degeneration of nerve tissues
(10) Abnormal overgrowth of bones

HOW VITAMIN A IS STORED IN THE LIVER

We often eat with our food more vitamin A than we need for our immediate requirements As has already been hinted, the excess is kept in reserve in a kind of store-house—in the liver This is very useful, for we are thus able to go

[1] Those interested in these medical aspects may consult a review by the writer in the *Annual Review of Biochemistry* (1935) pp 359 *et seq*

for long periods without any vitamin A in our food provided we have laid in a sufficient reserve. It is surprising how much can be put away in this manner One of my colleagues, Thomas Moore has given rats such large amounts of vitamin A that they may accumulate in their livers sufficient reserves to last them a theoretical period of 100 years—although the actual life span of a rat is only about five years! In practice, however various factors are found to influence the rate at which the vitamin is used up from the liver Thus it sometimes disappears quite rapidly Under other conditions its utilization and absorption into the liver from the blood may prove to be unsatisfactory Vitamin E (to be discussed in Chapter VIII) is one of the things which seems to assist in maintaining the reserves of vitamin A If the vitamin E in the diet is low any deficiency of vitamin A is thereby accentuated. Thus vitamin E can be described as a vitamin A-sparing substance

We shall return to this rather puzzling question of the safeguarding of the body's reserves later in the chapter (p 234)

VITAMIN A AND THE MOTHER

The young offspring receives its first supply of vitamin A either from the mother before birth, or through the milk. We can see therefore the necessity of giving the expectant or nursing mother ample supplies of vitamin A But it should be added that another of my colleagues, the late Dr W J Dann showed that the young animal does not receive any very great excess in this way from the mother it is important therefore to start giving vitamin-containing foods to the child immediately it is weaned.

THE CARROT PIGMENT CAROTENE, AND ITS CONNEXION WITH VITAMIN A

One of the most interesting developments in modern vitamin science was the connexion which was revealed between carotene, the yellow colouring matter of carrots (and many other natural foodstuffs) and vitamin A

We now recognize that vitamin-A activity is the joint property of two different and distinct types of substance (1) vitamin A proper a colourless

substance found only in the *animal* kingdom as in cod-liver oil and other liver oils and (2) carotene the yellow pigment, found *in many plant materials* and also together with vitamin A itself in butter and in egg-yolk.



Fig 75 Crystals of β-carotene, colouring matter in carrots. Primary vitamin A or pro-vitamin

VITAMIN AND PRO-VITAMIN

These two substances carotene on the one hand and the colourless vitamin A on the other are now seen by the chemist to be closely related one to the other [1] But, as usual, the truth was discovered only after long effort—after numerous investigations by a series of scientists in different parts of the world with some controversy on the way

The first step was in 1919 when Dr H Steenbock of the University of Wisconsin U S A. pointed out that the vitamin-A potency of certain plant products seemed to run parallel with their degree of yellowness. The yellower kinds of maize, for example, were more potent than the paler cereals Dr Steenbock suggested therefore that vitamin-A activity had to do with the

[1] There are several varieties of carotene, called α β- and γ- β-carotene is the most active. There are also at least two forms of vitamin A vitamin A_1 and vitamin A_2. See below p 222.

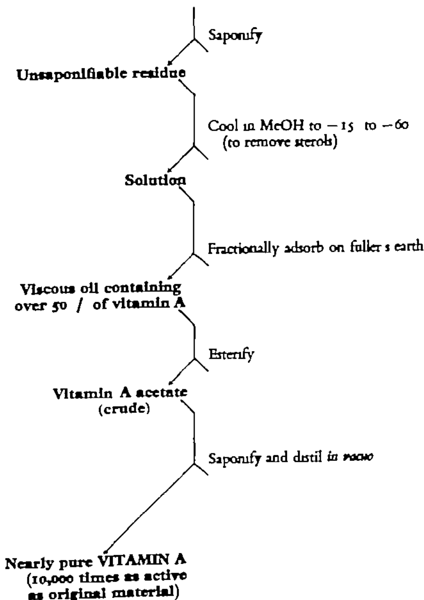
presence of the yellow colouring matters in question called the carotenoid pigments by the chemists He even got so near the full true explanation as to say that the vitamin A of animals might be a colourless variation (or leuco-form) of carotene. After a time however the theory was generally abandoned because it was stressed by others that vitamin A from animal sources seemed to have no connexion with yellowness For example Dr L. S Palmer in the United States found that by feeding hens on diets containing no carotene pigments he was able to get them to lay eggs the yolks of which were free of any yellow colour and from such eggs normal chicks could be hatched. If vitamin A was a yellow substance, as suggested how did the chicks survive without it? Again the late Dr Marjory Stephenson of the Biochemical Laboratory in Cambridge found that she could take butter and remove all the carotene from it by chemical means and it still cured xerophthalmia in rats—that is, it still contained vitamin A That seemed clear enough evidence against the yellow colouring matter being the vitamin. It could not be foreseen at the time that there were these two separate and distinct forms of vitamin A the colourless and the coloured, and that by a particularly unfortunate coincidence the two products studied, egg-yolk and butter are almost the only two natural foodstuffs known (see p 218) that happen to contain both of them owing their potency partly to one and partly to the other

Nearly ten years later in 1928 Professor Hans von Euler in Stockholm was struck by the fact that materials known to be rich in vitamin A gave certain chemical colour tests similar to those displayed by carotene He therefore tried feeding carotene to vitamin-A deficient rats to see if it was active, and found that in fact it was He rightly reached the conclusion that vitamin-A activity must be the property of the *peculiar double-bond system* presumed to be present in the molecules both of carotene and vitamin A

The problem was taken up by Dr Thomas Moore my colleague at the Nutritional Laboratory Cambridge It was he who found the true explanation namely that the yellow carotene and the colourless or classical vitamin A of liver oils are two distinct substances when carotene is fed to an animal it becomes converted in the body into vitamin A In the latter form it is stored up in the liver

TABLE LXI *Process for concentrating vitamin A*

(After Karrer and others)



It may be that the liver or some other organ in the body—more probably (as now appears) the wall of the gut—contains a ferment, named in advance *carotenase* responsible for converting the carotene which we eat into the vitamin A which our bodies need. But no one has yet succeeded in isolating this hypothetical ferment

CONCENTRATING VITAMIN A

This natural vegetable pigment carotene, had been isolated by the chemists a good many years previously long before its vitamin activity was in any way suspected. (This reminds us of the history of vitamin C and of nicotinamide pp 113 88.) Work was still in progress however towards the isolation of the colourless form of vitamin A from liver oils Many workers had helped in turn including Steenbock and others in America, Takahashi in Japan Drummond & Heilbron as well as Moore in England and Karrer in Switzerland. In this way by about 1934 vitamin A had been obtained almost pure, that is to say with less than 10 per cent of impurities. The process involves first, removing the main fatty part of the oil (the vitamin is found in the non-fatty or waxy residue) and then separating the vitamin from the waxy constituents The final stages include processes of adsorption and of fractional distillation The vitamin concentrate so obtained represents about 1 part in 2000 of the original fish-liver oil (see Table LXI) The dose needed to cure a rat is as little as 1/1000 of a milligramme (= 1/30 000 000 of an ounce)

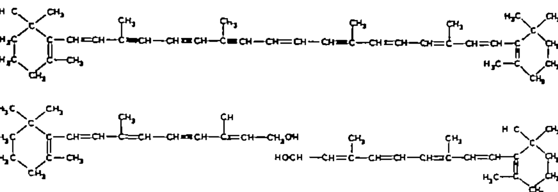
Finally in 1937 two biochemists in America, Holmes & Corbet succeeded in separating vitamin A in the form of colourless crystals from a concentrate such as this. In 1946–7 the artificial synthesis of vitamin A was accomplished, in the laboratories of organic chemists in Holland and in the U S A., and shortly afterwards in England

THE STRUCTURE OF VITAMIN A

Carotene is described chemically as a hydrocarbon that is to say it contains only hydrogen and carbon and no oxygen The arrangement of the atoms is shown in Table LXII What specially interests the chemist is the

arrangement of alternate double and single bonds along its long chain. It will be seen also that β-carotene has a perfectly symmetrical arrangement, with the same ring system at either end. This is called the *β-ionone* ring. In the long chain joining the two rings the same configuration of atoms will be seen to occur repeatedly four times over. It is called the *isoprene* group.

TABLE LXII *β-Carotene (above) and the two parts of vitamin A (vitamin A_1) derived from it (below)*



Vitamin A is derived from one molecule of carotene by splitting it into two at its middle joint, with the addition of the elements of water to each portion. In other words, while carotene is classed chemically as a *hydrocarbon*, vitamin A is an *alcohol*. This chemical relationship is also seen in Table LXII. The scientists principally concerned in unravelling these problems of chemical structure have been Karrer in Switzerland and Kuhn in Germany. Both have received the richly merited award of a Nobel Prize.

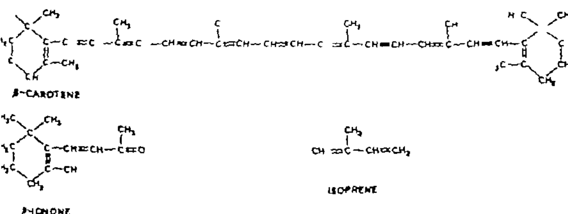
THE VARIOUS A VITAMINS AND PRO-VITAMINS

If we are to be precise, we must recall here that there are several slightly different forms of carotene. One variety, β-carotene, is far and away the most important. In many plant materials it is the only kind of carotene present in any appreciable quantity. Sometimes, however, smaller amounts of a second kind of carotene almost indistinguishable, and called α-carotene, are present, and occasionally there are traces of yet a third, γ-carotene. There

is moreover another closely related pigment called cryptoxanthin found in some red fruits and blossoms

All of these function as pro-vitamins in other words they are converted to vitamin A in the body and hence for the animal are equivalent to vitamin A (that is to say they are biologically active) Three of these four α- and γ-carotene and cryptoxanthin have approximately only one-half the vitamin-A value however compared with that of β-carotene itself weight for weight. The reason for this seems to be that β-carotene contains *two* of the peculiar β-ionone rings in its molecule whereas the other three substances have only *one* each—as shown in Table LXIV For a substance to have vitamin A activity the presence of both the *β-ionone* ring and of the *isoprene* group (Table LXIII) seems generally to be necessary

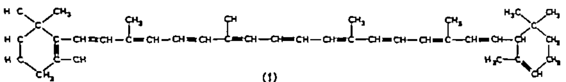
TABLE LXIII *Pro-vitamin A (β-carotene) and the chemical units on which its biological activity depends (β-ionone and isoprene)*

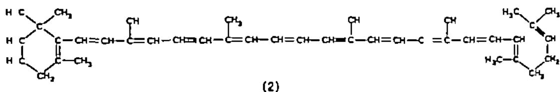


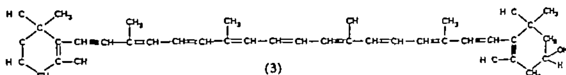
The student whose aim is to be comprehensive may wish to know that in addition at least five other somewhat rare, carotene pigments, which have been isolated by industrious chemists from various out-of-the way materials, have also been found to serve as pro-vitamin A They are however of little more than academic interest. Their names are given at the end of Table LXV

In contrast with the foregoing numerous other carotenoid pigments—natural colouring matters found in various vegetables and fruits—have no

TABLE LXIV *Chemical structures of some other pro-vitamins A (active carotenoid pigments)*







(1) *α-Carotene* (2) *γ-Carotene* (3) *Cryptoxanthin*

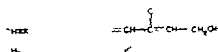
TABLE LXV *Vitamin-A active carotenoid pigments*

Name	Properties	
β-Carotene	*The most important.* Found in most plant material.	*Full activity*
α-Carotene	Found in smaller amounts in some plants	*Half activity*
γ-Carotene	Found in traces in some plants	*Half activity*
Cryptoxanthin	In some red blossoms and fruits	*Half activity*
Leprotene	In leprosy bacillus	*Of little practical importance*
Echinenone	In sex glands of sea urchins	*Of little practical importance*
Myxoxanthene	In certain algae	*Of little practical importance*
Aphanene, Aphanicene	In blue-green algae	*Of little practical importance*

vitamin A activity Such are *lutein* (in green leaves) and *lycopene* (in tomatoes)

When we turn to vitamin A itself we find a rather similar state of affairs As well as vitamin A proper or **vitamin A_1** which as we have learned is found in the liver oils of salt water fish there is also a second variant, named **vitamin A_2**, which has been detected in fresh-water fish In chemical structure it is almost identical with vitamin A_1 differing probably in an additional double bond in its ring structure (Table LXVI)

TABLE [illegible] *Vitam[illegible] [illegible]bove) and vitamin A_2 (below)*



MEASURING THE VITAMIN-A VALUE OF FOODS

Animal tests for vitamin-A activity are carried out in much the same way as described above for the other vitamins. Graded amounts of the food under test are fed to groups of rats and the effect is compared with that given by the international (League of Nations) standard. The first standard chosen, in 1934, was a preparation of β-carotene, and the international unit was then fixed by definition as the amount of vitamin activity present in 0·6 microgramme of this standard (a microgramme, it will be recalled, is the millionth part of a gramme and the latter about one-thirtieth of an ounce). In [illegible] it was decided to add an additional standard, namely one for vitamin A itself, as distinct from the old one for carotene (i.e. the pro-vitamin). This consists of a purified preparation of crystalline vitamin-A acetate. The new international unit corresponding to it is defined as 0·344 microgramme of the acetate, an amount calculated to contain exactly 0·3 microgramme of the pure vitamin A itself. Thus, we now have two standards, one for the vitamin and one for the pro-vitamin.

A difficulty in testing for vitamin A is that the rats taken for the test may be found to have immense but variable reserves of vitamin A stored up in their livers, depending on the amount of the vitamin that they have received in their past diets—maybe long before the experiment started. It would obviously be useless trying to test the effect of the quantity of vitamin A given in the test doses, if all the time the rat [illegible] to draw upon from their liver reserves. [illegible] precautions have to be taken. In [illegible] almost from the time of weaning [illegible] only a very small [illegible] Secondly, some time before [illegible] begins [illegible] containing no vitamin A [illegible] as shown by [illegible] Fig. [illegible] more [illegible]

already become so gravely ill from the deficiency probably with an infection supervening that all attempts at cure will fail and the test is frustrated.

The graded test-doses to be given must all be small, so as to be sufficient for no more than a partial degree of recovery of the rats. The exact degrees of adequacy or inadequacy can then be judged from the rates of the recovering growth curves. In this way the effects of given doses of the unknown are

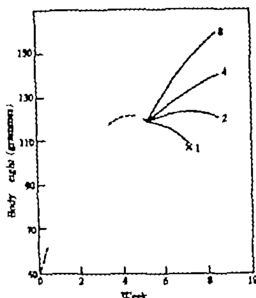


Fig 76. Cessation of growth of rats on a diet devoid of vitamin A (dotted line) followed by graded responses when graded doses of the vitamin are administered (in relative quantities of 1 2, 4 and 8)

compared with those of the standard. This method then is that of the *curative growth test* (Fig 76) Another possible alternative is a *preventive test* but this is difficult to do because of the dangers of variable reserves, already stressed. Yet again a totally different criterion of vitamin-A deficiency can be used instead of subnormal growth. The one found most feasible is to examine for the presence of the characteristic cornified cells, which, as we have learnt, are seen in the mucous membranes of an animal suffering from deficiency of the vitamin (p 209) These occur in the mucous membrane of the vagina, as well

as in other parts of the body and a useful test based on this fact is called t *vaginal-smear method*

Yet another procedure, if one wishes for example, to compare t vitamin-A values of different foods containing various active caroteno pigments is to administer these to rats and then to measure by means a chemical test (described in the next paragraph) the amounts of vitamin stored in the rats livers.

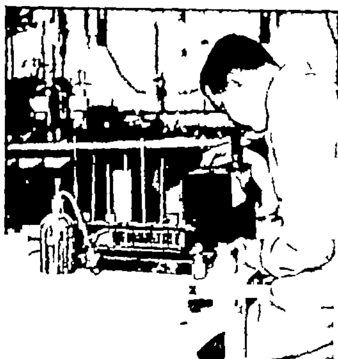


Fig 77 Measuring the vitamin-A potency of a foodstuff by the chemical colour test the 'tintometer' in use.

COLOUR TESTS FOR VITAMIN A

There are, then also chemical tests which can be used for the assay of vitamin A and which in certain circumstances may be even more accurate, and less time-consuming and therefore a great boon to the analyst. One method depends on the fact that the vitamin gives a transient blue colour when antimony trichloride is added to it under suitable conditions. To carry out

this test, the fatty portion of the food containing the vitamin is first treated with alkali to split up (saponify) the fat, and then the residue containing the vitamin is dissolved in chloroform. A given amount of antimony trichloride also in chloroform is next added. The more vitamin present the deeper the blue colour. The intensity of the colour can be estimated either by matching

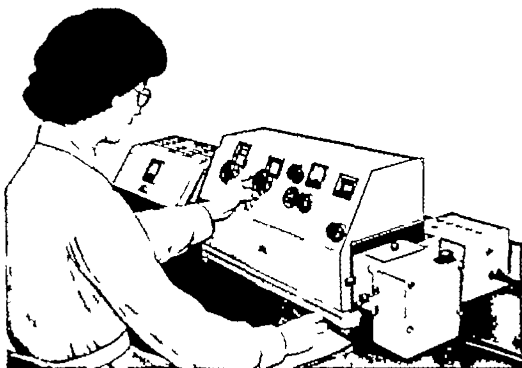


Fig. 78. Assay of vitamin A by the ultra-violet absorption test.
The photograph shows an appliance of modern design, a photo-electric spectrophotometer, as used for this purpose

it visually against a series of tinted blue glasses (Fig. 77) or preferably by electrical measurement. This test was adopted by the *British Pharmacopoeia* as the official method for assaying cod-liver oil for a minimum standard colour value.

The other test depends on the fact that the vitamin absorbs ultra-violet rays of a particular wave-length. It needs a special instrument, a spectro-

photometer (see Fig 78) When the intensity of absorption has been measured the result may have to be expressed in terms of equivalent to so much carotene (since carotene and not vitamin A was the standard originally chosen by the League of Nations) This is a matter of arithmetic, a particular *conversion factor* having to be used

ANALYSING FOODSTUFFS FOR CAROTENE

To measure the carotene (as distinct from the vitamin A) in fruit and vegetable foods a different chemical method has to be used. Since carotene itself is intensely yellow there is no need to add a reagent to it to produce a suitable colour for the chemist to measure. The natural yellow tint of the carotene itself can be measured, once it has been suitably extracted from the food. This may entail separation from certain other coloured products in the food, including perhaps other carotenoid pigments extremely similar to carotene but having no vitamin value. The way to *separate these natural pigments* from one another is to pass your extract through a so-called adsorption column or chromatogram (Fig 79) This consists of a tube closely packed with a suitable adsorbing material, such as di-calcium phosphate, or sodium carbonate The extract, dissolved in petrol, is made to flow *through slowly and steadily* and it is found that the different pigments get left behind on the filter bed at different stages in their passage, some earlier some later The adsorbent finishes its work of removing one pigment, for which it has some special affinity before it begins its job on the next for which it is less partial and so on in turn. Thus a series of separate coloured bands are formed, one

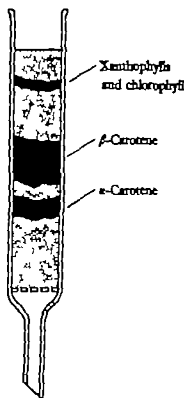


Fig 79. A chromatogram or adsorption column, as used for separating and measuring the amounts of the pro-vitamins A in foodstuffs.

The various pigments, some active and some inactive, separate out in different layers.

below the other one for each pigment sometimes it can be so arranged that the pigment the chemist is looking for is not collected in the column but is allowed to flow through the others being made to stay behind.

DEGREES OF AVAILABILITY

We now come to the rather troublesome point that so far as quantitative vitamin values are concerned the terms *vitamin A* and *carotene* are not precisely interchangeable or equivalent.

Compared with vitamin A itself the carotene present in foodstuffs is, on the whole less well utilized by an animal. For one thing the tissues of the vegetable food containing it, for example carrots, may be less completely broken up by digestion in the animal's alimentary canal. Some of the carotene in it therefore fails to get liberated for absorption. For another thing carotene being classed as a hydrocarbon—like paraffin, or vaseline—is itself probably more difficult of assimilation than vitamin A which is chemically an alcohol, a more soluble and reactive class of substance. Again, it appears that when carotene is dissolved in oil and administered in this way it is more fully utilized than when present in its native state in fresh or cooked vegetables. These may vary a good deal in the exact percentage of carotene absorbed from them and thus retained for use by the body Then again we must remember that the carotene after absorption has next to be mobilized and then converted into vitamin A. We do not know how the efficiency of this process may vary

Notwithstanding these complications, which may sound rather alarming at first hearing it is clear that dieticians need some fairly simple way of comparing the vitamin potencies of different foods, which may contain both vitamin A and carotene in varying amounts A rough-and-ready rule of sufficient accuracy for most purposes, is to express the biological potency of a food as equal to its vitamin-A content plus *one-third* of its carotene content. This makes allowance for the fact that weight for weight carotene is not so efficiently used in the body as is vitamin A. It neglects minor fluctuations from food to food which probably even themselves out sufficiently well when it is the whole diet which is being assessed.

WHICH FOODS CONTAIN VITAMIN A?

Carotene in foodstuffs, *the yellow pigment, is generally* (but not invariably) accompanied also by greenness, so that most green plants are rich in vitamin-A activity such are spinach, cabbage lettuce, Brussels sprouts and green peas. Certain yellow root vegetables, notably carrots and also artichokes and sweet yellow potatoes, are good Many colourless vegetable oils on the other hand are inactive. An exception is the deep orange-coloured, red palm oil of commerce, a staple product of parts of Africa and Asia, which is very high indeed in its carotene content.

Turning to animals, and therefore to vitamin A as distinct from carotene, the principal sources are the liver oils of fishes (notably halibut, and to a somewhat less extent cod) and of land animals (ox, calf, lamb etc.) Lard as ordinarily prepared loses its vitamin A in the course of manufacture.

Butter and eggs are distinctive in owing their vitamin-A potency partly to vitamin A itself and partly to carotene (p 218) The amount of the latter can vary considerably depending on the amount fed to the cow or the hen. This is a cause of the variation in the yellowness of butter and eggs. Milk is a moderately good source of A and of course its activity resides in the butter fat. Before the Second World War margarine in Britain was generally devoid of any vitamin A, but, as we have already had occasion to mention, war-time margarine was fortified, under government control, and has remained so since.

VITAMIN STABILITY AND INSTABILITY

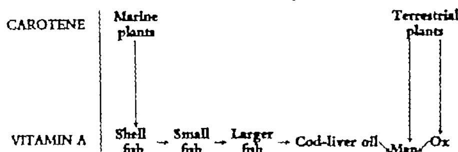
Vitamin A is fairly readily destroyed by oxidation especially when aided by heat. Fats kept warm may therefore lose their vitamin A fairly fast. A rancid fat rarely has any—rancidity being a token that oxidation *has occurred.* Another point is that certain substances present naturally in foodstuffs, *autoxidants* hasten the process of oxidation. Others again, *antoxidants*, retard it. One example of a protective substance of the latter kind is vitamin E (p 217) Here then we have the phenomenon of one vitamin aiding another and possibly being destroyed itself in the process—the oxidation of vitamin E taking place preferentially before that of vitamin A

THE BIRTH OF VITAMIN A

The origin of vitamin A is in the green things of the land and the sea carotene being synthesized by both marine and terrestrial *plant* life

Thus the ox gets his vitamin A from the carotene made by plants and we get our vitamin A either by eating the ox or else by eating the original carotene in the plant (Table LXVII)

TABLE LXVII *Man s source of vitamin A*



Similarly in the sea. The cod gets its vitamin A ultimately from the plant life of the sea, and there is an interesting vitamin cycle —or perhaps it would be more correct to call it a vitamin chain That is to say carotene made by the minute sea plants is eaten by shell-fish and other small creatures these are eaten in turn by larger fishes which form the food of still larger ones which are then devoured by the cod. Hence the vitamin in our cod liver oil.

> Big fleas have little fleas
> Upon their backs, to bite em
> And little fleas have lesser fleas,
> And so *ad infinitum.*

VITAMIN-A REQUIREMENTS

The presumed daily requirement of vitamin A for an adult, according to the scale recommended by the Health Commission of the League of Nations is 3000 international units (equal to – milligrammes of carotene or about 1/15 000 of an ounce) Such an amount is provided by about one pint of milk or by

an egg or by an ounce of butter or by a helping of greens. This figure agrees quite well with the results reached in tests carried out to determine the minimal requirements The method of test has generally been to measure the amount of the vitamin found necessary just to prevent people from developing the smallest degree of night-blindness or—what amounts to much the same thing—the smallest amount which will cure them when there is the slightest indication of some deterioration in their powers of dark-adaptation. Of course it is difficult to give a precise figure for this amount, because as already mentioned, carotene and vitamin A are assimilated with rather different degrees of efficiency and an ordinary diet generally contains both forms. In a report issued by the British Medical Research Council in 1949 it was stated that either 2600 international units of carotene or 1300 international units of vitamin A had been found sufficient to promote a slow cure of the signs of incipient vitamin-A deficiency in a group of human volunteers (the same conscientious objectors at Sheffield, already honourably mentioned in Chapter v p 152)

A further technical difficulty in assessing the requirements is that the amount of vitamin A mobilized from the liver or circulating in the blood, or dissipated, or utilized or excreted from the body may depend so much on other variables. Before closing this chapter it may be worth adding some final remarks on this latter rather tricky problem

VITAMIN-A EXCHANGES IN THE BODY

For example, it is found that to give particularly rapid cures of night-blindness, quite massive doses of vitamin A may be needed—much more than the ordinary reasonable daily intake. There is always the possibility that when a single such large dose is given there may be only a temporary cure of the night-blindness, soon followed however by a relapse to something near the original state It would seem that the eye has merely responded to the extra load of vitamin A being carried to it temporarily by the blood stream immediately following the big dose. When later this temporary flush in the blood has ceased and less vitamin is reaching the eye the production of the

visual purple naturally cannot keep up at the same pace, and vision deteriorates again In other words, the amount of vitamin A circulating at any given moment in a person s blood (which is what governs his performance in dark-adaptation tests) may possibly be merely the reflection of some recent particularly large helping of the vitamin and may not accurately indicate the state of the reserves in his liver or his general level of nutrition

The amount of vitamin A drawn out of the liver and sent circulating into the blood stream, for use in the body may in turn fluctuate according to various circumstances. For example it appears that a dose of alcohol or of benzidine can push up the amount of vitamin A despatched from the liver into the blood, and hence improve dark-adaptation The stores in the liver may also be protected or dissipated at varying rates as mentioned earlier Dr Thomas Moore has shown that, in animals a deficiency of vitamin E causes an increased rate of loss of the vitamin A from their livers (see p 217)

P C Leong in Singapore has recorded some interesting tests on dogs to ascertain what happened to the vitamin A in their liver and their blood when they were kept for a long time on a diet containing little or none of the vitamin Gradually month by month, the amount of vitamin A in their livers fell That in the blood quickly dropped to a very low level indeed, but it remained so for many months, before eventually definite symptoms of illness due to the deficiency began to appear

We mentioned in Chapters III and IV how tests on urine could be used to assess one s level of nutrition in vitamin B_1 or the pellagra-preventing vitamin. This cannot be done for vitamin A for normally there is no vitamin A in human urine—although it is excreted in certain diseased states Dog s urine curiously enough, contains vitamin A as a normal constituent.

NATIONAL NUTRITIONAL STATUS AS MEASURED POST-MORTEM

One way of determining the vitamin-A status of different large groups of the population, which has been employed by Moore is to examine at random the corpses of people killed in accidents in different areas of the country or in different social groups. Analysis of their livers shows whether their

vitamin reserves are high or low This has already been mentioned in another connexion on p 212 One of Moore's findings was the happy one that there was no deterioration in vitamin-A reserves as a result of the restricted war-time diets of 1939–45

DIAGNOSIS OF DEFICIENCY

To ascertain whether any particular individual is or is not below the optimum in his intake of vitamin A the most sensitive test known is that for dark adaptation mentioned on p 204.

For a somewhat more advanced degree of deficiency the physician can examine the eye, to ascertain whether the conjunctiva is normal or showing any sign of being dried up and thickened (xerosis) or whether *Bitot's spots* are present (p 200) If the tell-tale horny keratinized cells can be detected in a gentle scraping from the surface of the cornea of the eye, the diagnosis is confirmed.

A dried thickened appearance of the skin with presence of a scaly follicular eruption, mentioned on p *213* *is another fairly clear symptom of* vitamin-A deficiency

Tests on the blood to measure its vitamin-A content, may be rather misleading and difficult to interpret, for the reasons already stated.

VITAMIN ACTION

When an animal is given a very great *excess* of vitamin A, certain ill effects may follow including an extensive demineralization (*decalcification*) of their bones This can perhaps be contrasted with the *overgrowth* of the bony tissues which may result from a *deficiency* of the vitamin. Possibly this observation may give us in the future some clue about the mode of action of vitamin A *We have to admit, however that although we know a great* deal about the final effects of deficiency of the vitamin we know almost nothing about its more intimate chemical mode of action. To recognize the morphological changes which occur in the cells is *only the first step The next will be to try* and learn which is the particular chemical reaction in the body in which vitamin A intervenes

CHAPTER VIII

VITAMIN E DIET AND STERILITY

In 1922 Professor H. M. Evans and his colleagues at the University of California were studying the effects of diet on the reproductive function of rats. They found that although his rats were being given a diet containing all the vitamins then known they still failed to breed normally This meant that there must be some new vitamin needed for reproduction It was present in lettuce and in wheat germ for the addition of either of these foods to the rats diet restored their fertility In due course the new vitamin was called vitamin E. At much the same time similar observations were made by Dr H. A. Mattill of the University of Iowa and also by Dr Barnett Sure of the University of Arkansas.

Before we go further we may admit that perhaps it is a little misleading to call vitamin E the reproductive vitamin because, in fact, *all* the vitamins and not only vitamin E are needed for normal reproduction In the absence of any one vitamin the animal's health becomes gravely impaired and loss of reproductive power is generally included among the symptoms noted. But there is, nevertheless, some justification for the name for with lack of vitamin E the sterility is after all quite the most prominent symptom of the deficiency whereas with the other vitamins sterility is but one incidental feature Moreover with vitamin-E deficiency the sterility is found by pathologists to be of a very special and distinct type, not to be mistaken for the sterility resulting from any other cause

THE CAUSE OF THE STERILITY

In the female rat lacking vitamin E all seems to go well until a fairly late stage in pregnancy Then the young which she is carrying all die and are resorbed by the maternal organism. The effect is quite distinctive and may be described as a resorption gestation Now give the female some vitamin E, and she

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consists of a darkening or discoloration in certain organs of the body notably in the uterus (see Fig 81) The origin of this abnormal pigmentation is thought to lie in a degeneration of the fibres of the muscle-tissues of the body

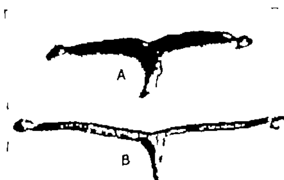


Fig 81 Abnormal pigmentation of the uterus, due to lack of vitamin E. Above uterus of a rat deprived of vitamin E. Below uterus of a normal rat for comparison.

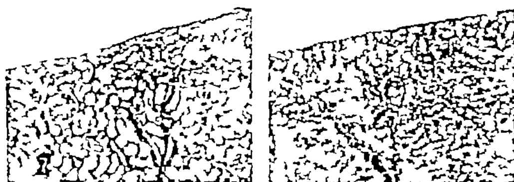


Fig 82. Effect of deficiency of vitamin E on microscopic structure of the kidney Left, rat deficient in vitamin E degeneration of convoluted tubules. Right, normal rat for comparison.

Thirdly characteristic changes can also be seen in the structure of the kidney to be precise degeneration of the epithelium of the convoluted tubules (Fig 82)

will be cured her next pregnancy will be successful and perfectly normal. In other words this form of female sterility is curable.

In the male rat, on the contrary the sterility is permanent and incurable. The male germ-cells degenerate and cannot be renewed.

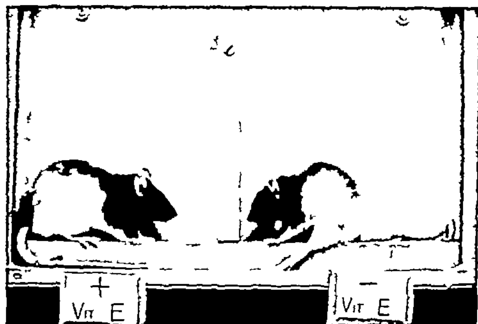


Fig 80. Muscular dystrophy in the rat caused by lack of vitamin E.

The rat on the left has received vitamin E and is normal. The rat on the right has been deprived of vitamin E and it can be seen that he is unable to make proper use of the muscles of his leg

OTHER SYMPTOMS OF DEFICIENCY

It would be a mistake, however to suppose that the ill-effects seen in rats deprived of vitamin E are limited only to these abnormalities in their reproductive function. Other symptoms of deficiency were discovered soon afterwards.

First, there is often a wasting and weakness of the muscles the rat is unable to hold himself in his normal posture he droops and fails to use his limbs properly Fig 80 illustrates this condition of muscular dystrophy

A second symptom noted by my colleague Dr Thomas Moore and others,

consists of a darkening or discoloration in certain organs of the body, notably in the uterus (see Fig. 81). The origin of this abnormal pigmentation is thought to lie in a degeneration of the fibres of the muscle-tissues of the body.

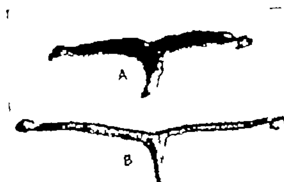


Fig. 81. Abnormal pigmentation of the uterus, due to lack of vitamin E. Above, uterus of a rat deprived of vitamin E. Below, uterus of a normal rat for comparison.

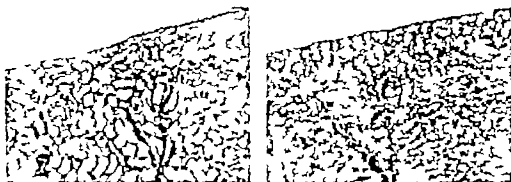


Fig. 82. Effect of deficiency of vitamin E on microscopic structure of the kidney. Left, rat deficient in vitamin E: degeneration of convoluted tubules. Right, normal rat for comparison.

Thirdly, characteristic changes can also be seen in the structure of the kidney, to be precise, degeneration of the epithelium of the convoluted tubules (Fig. 82).

The last symptom of deficiency to be mentioned here is one that has b
observed not so much in rats, but rather in *chickens* and in some other b
It consists of an accumulation of fluid or so-called exudate in various p
of the body This particular symptom was at one time often referred to as
exudative diathesis At first it was not recognized as being due to deficie
of vitamin E, but a special Anti-exudative-diathesis Factor was postulate
account for it The discovery that this anti-exudative diathesis factor is n
other than vitamin E means, happily, that in this instance at any rate the
one less vitamin to be added to our constantly growing vitamin alphab

(A curious symptom sometimes observed in rats deficient in vitamin
a depigmentation of the teeth Rats teeth are normally not white, but a
pale yellowish brown tint. In absence of vitamin E the natural pigmentat
tends to fade. This, however is not a very specific effect of deficiency
vitamin E for the same kind of dental depigmentation is observed also
rats deprived of vitamin A)

The symptoms are listed in Table LXVIII It ought to be added perh
that the reproductive failure still remains the most distinctive feature
vitamin-E deficiency

TABLE LXVIII. *List of symptoms of vitamin-E deficiency as seen in rats and some other animals*

(1) REPRODUCTIVE FAILURE
- (a) in female resorption gestation
- (b) in male degeneration of germ cell.

(2) Muscular dystrophy
(3) Degeneration of muscle fibres and discoloration in uterus and elsewhere.
(4) Kidney lesions (degeneration of epithelium of convoluted tubules).
(5) Exudative diathesis, encephalomalacia (crazy-chick disease)
(6) Dental depigmentation (also in vitamin-A deficiency).

TESTING FOR VITAMIN E

One characteristic feature about vitamin E is that as with vitamin A, reserv
of it can be stored with remarkable efficiency within the rat s body Instead
giving the rat one daily dose every day throughout the year it is as satisfacto

to give him 365 doses all on the one occasion on the first day of the year The rat will manage just as well

This principle is made use of in testing foodstuffs for vitamin E. You take rats which you know to be already short of vitamin E and which would

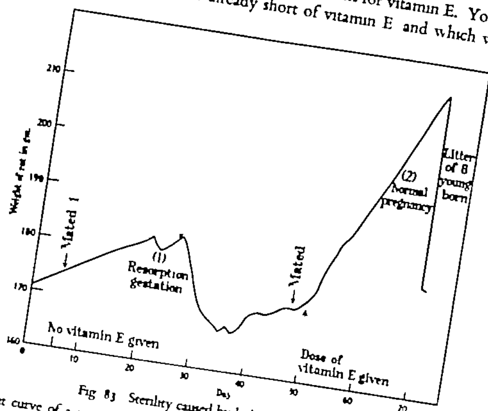


Fig 83 Sterility caused by lack of vitamin E.

Weight curve of a rat showing two pregnancies the first failing through vitamin E shortage and the second succeeding, vitamin E now being given.

In vitamin-E deficiency the rat lost weight gradually towards the end of the gestation period, owing to resorption of the foetuses. In the second, normal gestation, the body weight of the rat is seen to fall precipitously at the moment the young are born.

otherwise develop the characteristic reproductive failure (resorption gestations) and then you dose different graded amounts of the food and so find how much is needed to restore the rats—or 50 per cent of them—to normal fertility for a period of time long enough to carry them successfully through one pregnancy

For many years the only way to assay foodstuffs for their vitamin-E value was by means of such tests on rats Later as with other vitamins, chemical methods were gradually worked out. One such chemical test, due to Dr A. Emmerie in Holland, depends on the power of vitamin E to reduce ferric salts to the ferrous state. A chemical reagent called *dipyridyl* enables one to detect how much of the ferric salt has been so changed. A second test is quite similar but makes use of a gold salt in place of the ferric salt. Measurements are made either of a colour or of the electric potential in the solution.

FOOD CONTAINING VITAMIN E

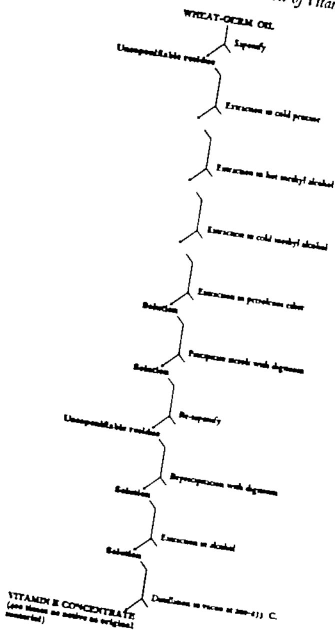
Green leaves and the embryo of seeds (e.g wheat germ) *are the best known* sources of vitamin E. Most other foods are less potent. It is remarkable that cod-liver oil so rich in two other important fat-soluble vitamins, namely vitamins A and D is devoid of vitamin E.

Certain *fresh* fats do *contain small amounts of vitamin E, but when the fat* becomes the slightest degree rancid, the rancidity rapidly destroys the vitamin. In fact, fat which is at all stale will destroy the vitamin in any other foodstuffs mixed in with it. It has been found that iron salts added to a food also tend to destroy *it* but apart from this the vitamin *is in many ways* remarkably resistant and less easily inactivated than are some of the other vitamins for example, it can be heated safely to a remarkably high temperature, or treated with acids or alkalis, or subjected to hydrogenation processes, etc.

CONCENTRATION OF VITAMIN E

The process (Table LXIX) used for concentrating vitamin E is similar to that for vitamin A except that we start with wheat-germ oil as the raw material. The main fatty part of the oil is first removed, and the vitamin E is then found to be left in the residue Next the waxy material (sterols) can be removed from the latter leaving the vitamin behind and then—after a number of further operations—a portion rich in vitamin E can be prepared by distilling the residue over in a vacuum, at 200°C. This distilled vitamin E

TABLE LXIX *Concentration of Vitamin E*



concentrate, which is 400 times more potent than the original raw material, contains only the elements carbon, hydrogen and oxygen and no nitrogen or sulphur (Compare it, for example, with vitamin B_1 which contains both nitrogen and sulphur)

ISOLATION OF VITAMIN E

Early attempts at isolation based on such methods proved very laborious and difficult, as animal tests lasting several weeks had to be carried out after each operation, to see what had happened to the vitamin i e. in which fraction it was to be found. But, finally in 1936 fourteen years after the start of his earliest experiments Professor Evans was able to announce at last the separation of what appeared to be the vitamin itself in a pure state The final step in the purification involved treatment with cyanic acid and the separation of a solid *allophanate*

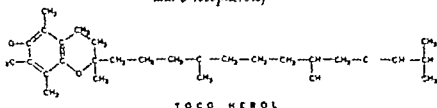
Chemically speaking vitamin E was found to be an alcohol and its formula was ascertained to be $C_{29}H_{50}O_2$ At the suggestion of Professor George Calhoun Evans gave the pure substance the name α-tocopherol —from *tokos* = childbirth *phero* = to bear *-ol* indicating an alcohol. A second form differing only very slightly from the first, was named β-tocopherol.

CHEMISTRY OF VITAMIN E

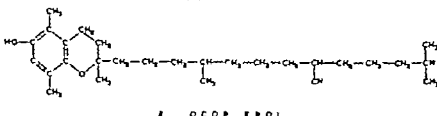
In due course the exact chemical nature of these two forms of vitamin E was established and yet a third form and then a fourth, named γ-tocopherol and δ-tocopherol respectively were isolated.

β-Tocopherol was found to be slightly more active than α-tocopherol and several related substances have since been synthesized artificially by organic chemists and shown to have some degree of activity The chemical relationships of vitamin E are shown in Tables LXX and LXXI. Various forms of it are now prepared industrially either from natural sources, such as cotton-seed oil, wheat-germ oil or maize-germ oil, or by synthesis.

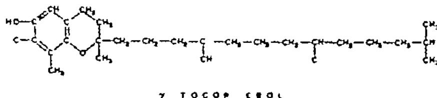
TABLE LXX *Chemical structure of the four forms of vitamin E (α- β- γ- and δ-tocopherols)*



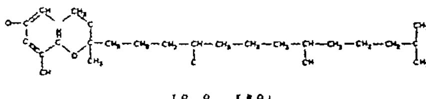
TOCO HEROL



OCOP EROL



γ TOCOP EROL



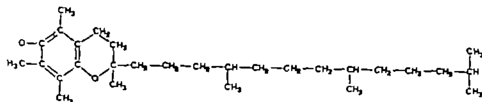
TO O EROL

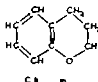
α-tocopherol = 5 7 8-trimethyl-tocol
β- = 5 8-d
γ " = 7 8-di
δ- " = 8-mono

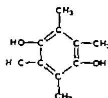
HUMAN APPLICATIONS

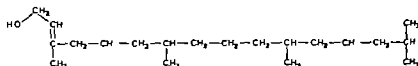
Naturally one is more interested in the causes of sterility in man than in rats Is vitamin E medication likely to be of use in overcoming childlessness in human beings? So far there is insufficient conclusive evidence either one way

TABLE LXXI *Chemical relations of α-tocopherol (vitamin E) showing how it is derived chemically from duroquinol and phytol and its relation to chroman*









or the other and most experts would regard the matter as still *sub judice* Vitamin-E deficiency has been studied most intensively in the rat and mouse, and other species still await fuller investigation. It is true, however that claims have been made that cases of habitual abortion in cattle in certain districts were effectively cured by vitamin E and some expert veterinarian opinion has been inclined to view the claim as a true bill

But, all things considered, many vitamin specialists would probably argue that any severe lack of vitamin E is unlikely to be widely prevalent among human beings certainly in this country They would maintain that the vitamin is fairly widely distributed in natural foods and relatively small

amounts of it would appear to be sufficient to maintain fertility Other witnesses called against vitamin E might then go on to maintain that, if it is admitted at all that lack of fertility in humans can sometimes, and in some regions, be attributed to dietary errors, then a lack say of vitamins A or B_1—deficiencies of which are still only too common in many parts of the globe—would be more likely to be the responsible factor

VITAMIN E AND HABITUAL ABORTION

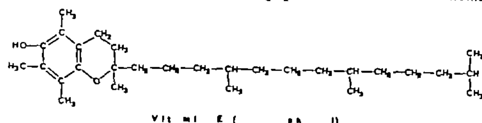
However an impartial judge would have to rule that this is only one side of the picture. The possibility that some cases of sterility in women are due to vitamin-E deficiency cannot be entirely discounted A diet consisting largely of white bread (such as was usual in Britain before the Second World War) would admittedly be low in vitamin E. Dr Vogt Møller in 1931 reported that several women who had had four or five successive miscarriages were cured, and had normal pregnancies after vitamin E had been administered to them He and other clinicians later published accounts of the successful use of vitamin E in similar cases of habitual abortion

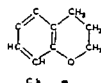
Against this, it has been argued that the women might, for all we know have done just as well and ceased to have miscarriages, without any vitamin E at all. (In the same way as, according to the old Spanish proverb a cold treated by the doctor can be cured in a fortnight, whereas if left to itself it will persist for at least two weeks!) Indeed as the critics remind us, after one or more previous miscarriages, the chances of a cure —i.e. of later pregnancies being successful—are still very good, even without any treatment. See Table LXXII after Dr P Malpas. The critics would therefore

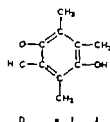
TABLE LXXII *Cure of habitual abortion in women without treatment*

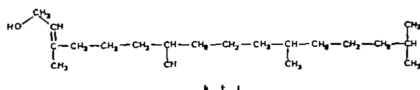
No of previous miscarriages	Chances of next pregnancy ending successfully (%)
1	78
2	62
3	27
4	6

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contend—as one has playfully put it—that results just as good as those attributed to vitamin E might have resulted from treatment, with (*a*) shall we say *cold baths* or (*b*) *no cold baths* or (*c*) *spiritual healing* or (*d*) by instructing the patient to lead a perfectly *normal life* or (*e*) alternatively insisting on a specially *sheltered existence*!

As against those sceptics, however Mr A. L. Bacharach, from a statistical study of the records has argued that the results of treatment of habitual abortion in women by vitamin E are altogether too good to be attributed to mere chance.

Probably the only way of settling the matter once and for all, one way or the other and convincing everybody will be by a controlled trial. That is to say there must be an equal number of negative controls For every case of habitual abortion treated with vitamin E every second case must be left without extra vitamin E This raises ethical issues, for if we are satisfied that vitamin E is of any value we cannot withhold it. In any case all this will take time and many more tests will have to be made before it will be possible to assess at all accurately just how far vitamin-E deficiency is a common cause of habitual abortion in women.

OTHER CLAIMS FOR VITAMIN E

Since one symptom of vitamin-E deficiency in animals is a condition of muscular dystrophy it was natural for clinicians to undertake a series of trials to ascertain whether any types of muscular dystrophy and similar disorders as seen in their human patients would respond to treatment with vitamin E. The results have been disappointing and there seems little doubt that human muscular dystrophies cannot generally be due to simple lack of vitamin E.

One cause for thankfulness perhaps is that experiments on animals have clearly proved that when *extra* vitamin E is given, the fertility *is not increased beyond the normal limits*!

CHAPTER IX

VITAMIN K AND BLOOD CLOTTING

A new chapter appears in this edition devoted to vitamin K It merits a chapter to itself because vitamin K is one of the few newer vitamins which has already found an important use in clinical medicine. (Another is vitamin B_{12} to be discussed in Chapter XI)

A BLEEDING-DISEASE OF BIRDS

The history of vitamin K begins in 1935 when Dr H Dam at Copenhagen described a new disease in chicks, which he had observed when they were kept to a diet of cereals and yeast. The special feature of the disease was the occurrence of bleeding in different parts of the body These haemorrhages, as he found were due to the blood taking too long to clot. When certain natural foodstuffs, particularly green leaves, were once more restored to the diet, the birds recovered and the blood now clotted in the normal time. Dam therefore called the new vitamin the blood-clotting vitamin or the coagulation factor —*Koagulation Faktor* in German or Danish. This term became shortened to the K factor and hence vitamin K.

THE CAUSE OF THE BLEEDING

Very soon afterwards in 1936, a colleague of Dam at Copenhagen, named Schønheyder discovered the reason why the blood takes too long to clot in this bleeding-disease in chickens It was simply that there was an inadequate supply in the blood of a particular enzyme (ferment) called prothrombin which as was already well known, plays an important role in the clotting of blood, and hence in the prevention of haemorrhages. When some foodstuff containing the new vitamin was given to the birds, the prothrombin value became normal again hence the blood was able to clot in the normal time and the haemorrhagic disease was cured.

This effect of vitamin K on prothrombin, and hence on clotting time, is really the fundamental point about its mode of action and little more than that is known. We cannot yet describe in detail how the vitamin is able to change the prothrombin value. The two substances prothrombin and vitamin K are not simply interchangeable that is vitamin K itself cannot function as prothrombin, nor has prothrombin, as such, any vitamin K activity All we can say is that vitamin K is necessary in some way for the normal formation in the liver of prothrombin the blood-clotting ferment. (The latest researches by Professor Dam, published while this edition is in the Press incriminate a particular component of the complex prothrombin blood clotting system named the delta factor the formation of which is impaired in vitamin-K deficiency)

IN OTHER SPECIES BACTERIAL SYNTHESIS

Soon after the original discovery of vitamin K it was found that a vitamin-K deficiency-disease could be produced also by similar means in other birds as well as in chicks—geese ducklings and so on. It was not possible, however to get the symptoms at all readily in mammals e.g rats mice, etc. The reason, which only afterwards transpired, is that in the higher animals vitamin K is under ordinary circumstances manufactured by the bacterial population in the intestine. *Hence the host has a constant supply of vitamin K* available even when none is given in the food.

TESTING FOODS FOR VITAMIN K

To measure the amount of vitamin K in different foodstuffs, tests were carried out on chickens, the index used being of course the clotting power of their blood. Four hours after a deficient chicken had been given a foodstuff containing vitamin K, it was shown, the clotting time had already returned to normal. *One exact procedure for the quantitative assay of vitamin K in* foodstuffs which was then developed was to administer it in graded doses to chickens and then after three days to determine the prothrombin value of their blood.

Vitamin K is found to be fairly widely distributed in foodstuffs being present in the fatty portion of various animal and vegetable tissues It is thus a fat-soluble vitamin like vitamins A D and E

Green leaves are especially rich in it, lucerne (*alfalfa* to the Americans) having often been used as the raw material for its preparation. Another exceptionally good source is fish-meal It should be noted that there is however very little of the vitamin in wheat-germ oil (which is so rich in vitamin E, p [illegible]) or in fish-liver oils (rich in vitamins A and D but not E, pp 186 23[illegible] and [illegible])

THE ISOLATION AND SYNTHESIS OF VITAMINS K_1 AND K_2

The investigator most prominently concerned in unravelling the chemical nature of vitamin K was Doisy of the University of St Louis U S A In 1939 he and his co-workers had shown that the two forms of vitamin K, called K_1 and K_2 present in natural foodstuffs, were both *naphthoquinone* derivatives This was a discovery of fundamental importance Naphthoquinone is a substance which was formerly of little interest to physiologists or biochemists but had been used as a reagent in analytical chemistry

Several other organic chemists in America notably Drs Almquist & Klose of Berkeley and Drs Fieser Campbell & Fry of Cambridge, Mass and also Dr Karrer in Switzerland contributed vital information in this same year 1939 The first mentioned, Almquist & Klose made the remarkable observation that another already known naturally occurring naphthoquinone derivative named phthiocol (so-called because of its presence in the human tubercle bacillus) possessed vitamin-K activity

phthiocol = 2-methyl-3-hydroxy-1 4-naphthoquinone

Fieser and his colleagues worked out the chemical constitution of vitamins K_1 and K_2 already isolated from foodstuffs, with the following result

K_1 = *2-methyl-3-phytyl-1,4-naphthoquinone*

K_2 = *2-methyl-3-difarnesyl-1,4-naphthoquinone*

In 1940 Doisy and his co-workers accomplished the chemical synthesis of both K_1 and K_2 and of various other closely related artificial substances

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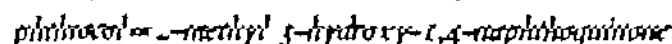
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THE ISOLATION AND SYNTHESIS OF VITAMINS K_1 AND K_2

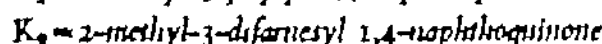
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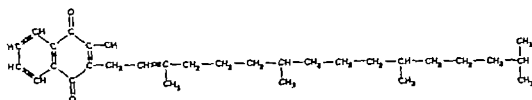

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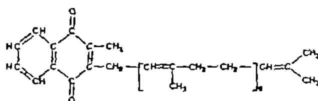
In 1940 Doisy and his co-workers accomplished the chemical synthesis of both K_1 and K_2, and of various other closely related artificial substances.

(many of which had vitamin-K activity) and this chapter of chemical endeavour very fittingly closes with the joint award to Dam and Doisy of the Nobel Prize for Medicine in 1943

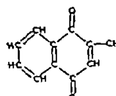
TABLE LXXIII. *Chemical structure of the K group of vitamins*



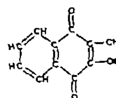
Vitamin K_1



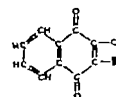
Vitamin K_2



Vitamin K_3



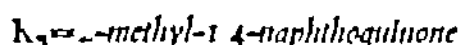
Phthiocol



General formula

CHEMICAL CRITERION OF BIOLOGICAL ACTIVITY

Three substances possessing vitamin-K activity have just been mentioned viz. K_1 K_2 phthiocol The common feature of these, and of other K-active substances, is that they are all derived from the same parent substance namely



This parent substance has itself a full degree of vitamin-K activity and is indeed preferred for medical use to the natural forms K_1 and K_2 since it is easy to manufacture synthetically and since it forms soluble derivatives which are more easily assimilated by the body The organic chemist will *readily understand that the long side-chains in vitamins K_1 and K_2 have the* disadvantage of making them relatively insoluble and so badly absorbed. One point of passing interest is the presence in K_1 of the *phytol* side-chain which is present also in vitamin E (p 246) and in chlorophyll the green colouring matter in the leaves of plants

ANTI VITAMIN K AND THE SWEET CLOVER DISEASE OF CATTLE

Perhaps one other fact is worth mentioning in regard to the chemistry of vitamin K and that is the existence of an anti-vitamin. We have referred to other anti-vitamins, while discussing nicotinamide (p 97) and vitamin D (p 189) Knowledge of anti vitamins is a relatively new development in nutritional science They may be defined as substances which, generally because of their close structural relationship to the vitamin itself or sometimes for some other special reason are able to block the vitamin or compete with it, and thus prevent its functioning

In the case of vitamin K a disease had been observed to occur naturally in cattle fed on pastures containing much sweet clover It was thus called the sweet clover disease, and was eventually found to be caused by a constituent in the plant, named dicoumarol which because of its similar chemical structure to vitamin K antagonizes it in the body and so produces a condition of vitamin-K deficiency—marked as usual by an insufficiency of prothrombin

in the blood, and in consequence by haemorrhages throughout the body If enough vitamin K is given to the cattle the *anti-vitamin is overpowered*, as it were, and the sweet clover disease is cured.

The anti-vitamin itself may have a possible use in medicine. Just as vitamin K is administered to ensure blood clotting so conversely when it is desired to prevent a clot forming—namely to guard against thrombosis—anti-vitamin K (dicoumarol) may be employed. But it is not without its dangers to wit, the risk of inducing haemorrhage, if too much is given

MEDICAL USES OF VITAMIN K

From what has already been said it will be understood that vitamin K is useful only for preventing, or curing those kinds of haemorrhagic diseases which are due specifically to the prothrombin level of the blood being too low—and in *no other* haemorrhagic disorders Thus vitamin K is not an anti-haemorrhagic cure-all For example, it is of no value in the well-known ailment of bleeders *haemophilia*—that inherited disease common in some of our European royal dynasties, occurring only in the male members of the family but transmitted to the males through the females

The three conditions in which vitamin K has been found of value, because *it corrects the low prothrombin which causes them* are

(1) in a haemorrhagic disease of the new-born

(2) to control haemorrhage after surgical operation for obstructive jaundice,

(3) in a conditioned deficiency of vitamin K i e one caused by intestinal obstruction or by failure to absorb it.

We may now turn to discuss these three categories in a little fuller detail

VITAMIN K AND HAEMORRHAGIC DISORDER OF THE NEW-BORN

Frequently in new-born infants a *transient fall in the level* of the prothrombin in the blood is found to occur in the first two or three days after birth It may persist until the fifth or sixth day and is the cause of a not uncommon condition—a so-called haemorrhagic diathesis seen in babies from two to

six days after birth Bleeding may occur in various parts of the body in the alimentary tract (as evidenced by the passing of discoloured stools) from the umbilical cord from the nose or palate or in the genito-urinary organs. (Some clinicians believe that haemorrhages within the skull too may sometimes have this same origin)

The supposed reason for the low prothrombin and the consequent haemorrhage is of interest. Ordinarily as we have learned (p 250) vitamin K is made for us by microbes in our intestine. In the new born infant, however the right types of microbes have not yet had a chance to become established as enough food has not yet passed into the intestine to support them Before birth the baby had been protected, since a certain amount of vitamin K had been able to pass from the mother to the infant within the womb

VITAMIN K IN THE PREVENTION OF NEO-NATAL HAEMORRHAGE

Remarkable success has accompanied the use of vitamin K in controlling this haemorrhagic condition in the new-born The most favoured procedure now widely followed in the United States, has been to administer vitamin K as a preventive—*to the mother* before the baby is born For one month prior to the calculated time of delivery 1 milligramme per day of the simple synthetic form of vitamin K (K_3 = 2-methyl-1 4-naphthoquinone, or menaphthone p 253) is given to the mother This has been found to be a remarkably efficacious way of preventing any lowering of the prothrombin and consequent haemorrhage So much so that this pre-natal treatment of the mother has become a recognized routine in some American clinics

In the absence of such pre-natal treatment, vitamin K is still found to be a valuable safeguard if given to the mother at the onset of labour

Yet a third alternative is to give the synthetic vitamin K (menaphthone) to the infant as a prophylactic at birth Drs Waddell & Lawson at the Department of Medicine Virginia, reported that of 400 such infants treated with vitamin K only 1 per cent developed haemorrhage whereas of .19 not so treated 10 per cent developed haemorrhages. Such results need no further comment

If neither the mother nor the child has received preventive treatment, and vitamin-K deficiency—low blood prothrombin and haemorrhages—should result, it is still not too late to institute *curative* treatment although all will agree that *prevention* is better The child is given 1 milligramme of synthetic menaphthone by injection or by mouth every 12 hours and the haemorrhages should thereby be brought quickly under control

VITAMIN K AND OBSTRUCTIVE JAUNDICE

So much for the new-born infant. The second important use of vitamin K in medical practice is in the prevention of haemorrhages after surgical operation for obstructive jaundice. This is an important advance in medical science but what is the rationale underlying it?

The explanation is simple enough obstructive jaundice consists of a blockage of the bile duct this blockage prevents the bile from flowing normally into the intestine it is driven instead into the blood stream, as the patient s greenish appearance clearly shows. Now the bile contains certain emulsifying agents which should be exercising their function in the intestine by aiding in the absorption of the fatty components of the foods we eat, including the fat-soluble vitamins and especially—as it concerns us at the moment—vitamin K. Since in obstructive jaundice there is an absence of bile from the intestine, vitamin K is not adequately absorbed Therefore, the patient becomes deficient in vitamin K, and a low prothrombin value results Hence the blood loses its power to clot normally and there is a tendency to excessive haemorrhages. Thus, when the surgeon operates, to remove the obstruction causing the jaundice, there is the risk of an excessive loss of blood since it takes too long to clot.

Formerly such haemorrhages supervening after operations for obstructive jaundice caused a high mortality rate The risk has now been largely banished vitamin K is given as a matter of routine before the operation. This corrects the deficiency restores the prothrombin level, and hence brings the blood-clotting time to normal.

At first when this treatment was new and only vitamins K_1 and K_2 (p 253)

were available it was necessary to give them by injection and also to inject bile salts simultaneously so as to ensure their being absorbed into the body. Now, however, the more soluble synthetic derivative already mentioned more than once, menaphthone, is used instead and so this problem of mal-absorption no longer arises.

Remarkable statistics can be quoted illustrating the success which has attended the use of vitamin K in controlling this risk of bleeding and hence the danger to life after operations for obstructive jaundice.

CONDITIONED DEFICIENCY

It is not only shortage of bile which may cause faulty assimilation of vitamin K. A similar conditioned deficiency may result when for any other cause there is a faulty absorption of fats (and in this term we include the fat-soluble vitamins) or indeed if there is any kind of internal obstruction interfering with assimilation of nutrients in general. Deficiency of vitamin K of this kind—manifested as always by low prothrombin value and haemorrhages—has been noted particularly in association with the three following types of disorder: (*a*) *steatorrhoea*, (*b*) *sprue*, (*c*) *intestinal obstruction*. The first mentioned, as its name implies, means that fats are being passed through the alimentary canal and excreted from the body instead of being absorbed. The second, sprue, is a disorder generally of tropical origin, also characterized by faults in fat absorption. The last-mentioned is self-explanatory.

In such cases of 'conditioned' vitamin-K deficiency once again treatment consists of administration of vitamin K—1–4 milligrammes of menaphthone per day. This is the microscopically small dose which may make the difference between a fatal haemorrhage and the saving of life. Truly a noteworthy achievement, the more so when we recall that until Dam's observations on chickens in 1935 no one had guessed the existence of such a thing as an anti-haemorrhagic, blood-clotting vitamin.

CHAPTER X

THREE ADDITIONAL B_2 VITAMINS

RIBOFLAVIN AND HUMAN 'CHEILOSIS' PYRIDOXIN AND 'RAT PELLAGRA' PANTOTHENIC ACID AND 'CHICK PELLAGRA'

In Chapter II and again in Chapter IV mention was made of three vitamins which are often found in foodstuffs alongside one another and accompanying the pellagra-preventing vitamin and which were each in turn mistaken at one time or another for the true P-P factor

It will be recalled that these three are

(1) *riboflavin* lack of which from the diet causes in humans a disorder known as cheilosis an inflammation on the lips

(2) *pyridoxin* or vitamin B_6 deficiency of which is responsible in rats for a severe skin disease, formerly miscalled rat pellagra

(3) *pantothenic acid* preventing a disease in poultry formerly known as chicken pellagra

It is now recognized that in various other species also these vitamins are of importance—e.g riboflavin for poultry dogs, rats and pigs and pantothenic acid likewise for poultry dogs, rats and pigs.

But whether these three vitamins are of great moment in practical human dietetics is still uncertain although they are of much scientific interest to physiologists and biochemists. We shall do no more here than give a brief *résumé* of some outstanding points about these three. For a still briefer summary see Table LXXXI at the end of this Chapter

I. RIBOFLAVIN AND HUMAN CHEILOSIS

As has already been mentioned, riboflavin is a yellow-coloured substance soluble in water the existence of which was first noted as long ago as 1879 by an English public analyst named Wynter Blyth. Like some other vitamins

vitamin derivatives (e g thiochrome, p 64) previously referred to it the typical property of *fluorescence* that is to say it emits a pale glow when a violet rays fall on it. Wynter Blyth called the substance *lactoflavine* ginally spelt with a terminal -e) lacto because he had found it in milk, vine indicating that it was coloured. Later substances which were

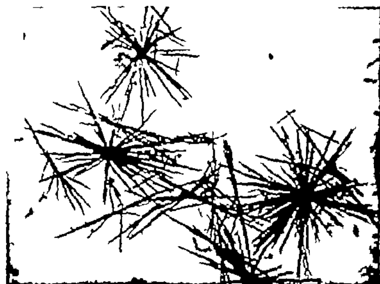


Fig 84. Crystals of riboflavin.

parently very similar or identical, were found to occur also in eggs, liver ney and other animal and vegetable tissues and were accordingly called *flavine hepatoflavine* etc

After Kuhn Gyorgy & Wagner Jauregg in Heidelberg in 1933 had nd that lactoflavin was a growth-promoting vitamin for rats (p 83) it came recognized that lacto- ovo- and hepatoflavins were really all one l the same substance and a new name riboflavin was therefore given— so to indicate that a peculiar chemical property of the substance is that contains a special sugar-like grouping *ribose* or to be more precise, *ribityl* 263)

RIBOFLAVIN DEFICIENCY IN MAN

Symptoms due to lack of riboflavin have been seen in human beings in America and elsewhere There are several special features Most notable perhaps is a severe inflammation at the corners of the mouth. This is shown in Fig 85 It consists of a condition of scaliness leading to cracking and maceration and so in time to the development of deep fissures Since the edge of the mucous membrane at the corner of the mouth is affected, the condition can be described as an angular stomatitis Another name, *cheilosis* has lately been used to describe more particularly the inflammation on the lips themselves. Accompanying these disorders around the mouth there may also be an inflammation of the tongue, which assumes an abnormal reddish hue—the so-called magenta tongue

An additional symptom described by some observers occurs in the *cornea* the transparent, horny covering of the front of the eyeball. This becomes opaque and an excessive number of small blood-vessels can be seen in it, when it is examined microscopically However these changes in the eye are probably not very specific as a diagnostic sign similar abnormalities have sometimes been recorded in people not suffering from riboflavin deficiency

We are still somewhat uncertain about the significance of riboflavin in practical dietetics The requirement is considered to be about 1 to 2 milligrammes per person per day Any severe deficiency at any rate, must be uncommon in most regions of the globe.

RIBOFLAVIN DEFICIENCY AND CURLED-TOE IN POULTRY

For the poultry farmer however riboflavin deficiency has been found to be of practical importance Birds raised intensively on certain restricted rations have developed an ailment, called because of the characteristic symptom curled toe This can be prevented or cured by provision of foodstuffs containing an adequacy of riboflavin. For good egg production and hatchability of the eggs a sufficient intake of riboflavin-containing foods is also of importance.







Fig 85 Effects of a deficiency of riboflavin in man.

Four features are shown in these pictures (1) the cracked and inflamed tongue (2) the fissures at the corners of the mouth, (3) the inflamed and cracked lips, and (4) the inflamed eyelids, which are closed in spasm.

RIBOFLAVIN DEFICIENCY IN OTHER ANIMALS

Poor growth and other ill effects have also been observed in pigs kept on ill-balanced rations low in riboflavin

In dogs restricted experimentally to diets lacking in riboflavin, a deficiency disease occurs which because of its most striking feature, has been aptly called yellow liver

Rats deprived of riboflavin fail to grow and develop an eczematous kind of disorder of their skin and changes in the cornea similar to those recorded in man.

Finally riboflavin is an essential nutrient for many kinds of bacteria which in its absence lose their activity and fail to multiply This can be shown by feeding them on special media devoid of riboflavin.

CHEMICAL ROLE OF RIBOFLAVIN IN THE BODY

The secret of the mode of action of riboflavin (like that of vitamin B_1 or of nicotinamide, already described) is that it functions in a *co-enzyme* That is to say it forms one part of an enzyme (= ferment) which, when present in the merest traces, is able to bring about chemical changes in the body

The particular enzymes of which riboflavin is a component are called collectively the *flavoproteins* There are a considerable number of these—at least ten—but they all resemble one another in being concerned in certain chemical reactions in the body which involve the *transport of hydrogen* atoms.[1] Thus in one enzyme, riboflavin is concerned in transporting hydrogen atoms to a substance which we may call A in a second enzyme to substance B and so on. A list of these ten or more substances A B etc. is given in Table LXXIV The difference between the enzyme which acts on substance A and that which acts on substance B is that the riboflavin in each is combined with a different protein molecule otherwise they are the same. In other words, it is the variable protein molecule in the flavoprotein enzyme which determines the chemical substance upon which it acts.

[1] Vitamin B_1 and nicotinamide as co-enzymes control other chemical reactions in which hydrogen has also to be transported.

This brings us to the chemistry of riboflavin and to the way in which it combines with the protein molecule and with certain other substances still to be mentioned to form an enzymic system

TABLE LXXIV *Some chemical events in living tissues initiated by riboflavin-enzyme systems*

Name of the riboflavin-enzyme system	Substances on which it acts
D-Amino-acid oxidase	D-Amino acids
Reductase	Fumaric acid
Diaphorase	Nicotinamide co-enzymes
Cytochrome reductase	Cytochrome
Aldehyde oxidase	Aldehydes
Xanthin oxidase	Hypoxanthine
Aceto-acetic decarboxylase	Aceto-acetic acid
L Amino-acid oxidase	L-Amino acids
Pyruvic oxidation system	Pyruvic acid

CHEMISTRY OF RIBOFLAVIN AND OF THE FLAVIN ENZYMES

The chemists to whom we are specially indebted for unravelling the chemical constitution of riboflavin and for its eventual synthesis, which was accomplished in 1935 were Kuhn in Germany and Karrer in Switzerland.

Riboflavin itself can be regarded as built up from two simple substances, the first called *iso-alloxazine* and the second, *ribose* (the sugar mentioned on p 259)

But before it can function in the body as an enzyme, riboflavin has first to join up with other substances, namely phosphoric acid and adenine to form a still more complicated structure The resulting compound is called

riboflavin-adenine-dinucleotide

Finally the latter joins with the specific protein to form a specific enzyme as already explained (See Tables LXXV LXXVI and LXXVII)

TABLE LXXV *Chemical structure of riboflavin showing its relation to the parent substance iso-alloxazine*
(*The full systematic chemical name for riboflavin is dimethyl-9-D-ribityl-iso-alloxazine*)

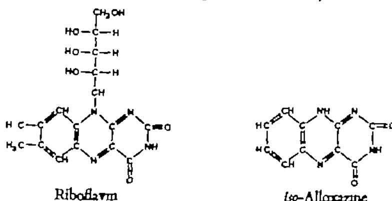


TABLE LXXVI *Steps in the building up of the riboflavin enzyme systems*
(See also next Table)

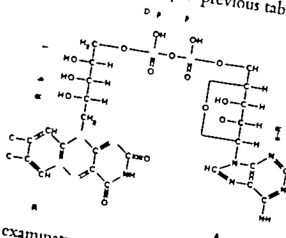
(1) *ISO*-ALLOXAZINE
↓ + ribose
(2) RIBOFLAVIN
↓ + phosphoric acid
+ ribose
+ adenine
(3) RIBOFLAVIN-ADENINE DINUCLEOTIDE
↓ + protein
(4) FLAVOPROTEIN

ESTIMATING RIBOFLAVIN IN FOODSTUFFS

The method most widely used for measuring the amounts of riboflavin in foodstuffs is one based on the fact mentioned on p 259 that it is a highly fluorescent substance. Before the fluorescence can be measured by an appropriate apparatus a preliminary extraction process has first to be applied to the food

A second procedure is to compare the growth-rate of rats which are given diets containing all other known vitamins except riboflavin Graded amounts

TABLE LXXVII *Structure of riboflavin-adenine dinucleotide showing its component parts* (Cf previous table)



of the food under examination and of pure riboflavin are added, and the results compared

A third test depends on the use of micro-organisms. Certain bacteria when growing normally produce *lactic acid* as an excretory or metabolic product. In the absence of enough riboflavin they fail to grow satisfactorily and the effect is detected by a chemical measurement of the lessened production of lactic acid.

Foodstuffs best as sources of riboflavin include milk yeast, liver kidney egg white green vegetables—and beer Riboflavin is however present to some extent in almost all foodstuffs, both animal and vegetable The growing plant is able to synthesize it the animal gets his supply by consuming either vegetable foods or other animals

II PYRIDOXIN (B_6) AND RAT PELLAGRA

This is the second of the three additional B_2 vitamins discussed in this chapter

The history of vitamin B_6 or pyridoxin (*alias* adermin) begins in 1926 when (as the reader will recall Chapters II and IV) Goldberger in the U S A. discovered a skin disease in rats which he thought to be identical with pellagra in human beings He called it rat pellagra At this time in the absence of

evidence to the contrary it was thought that there was only one B_2 vitamin—it was described as being heat stable, present in yeast needed to prevent both human pellagra and rat pellagra and required also for the growth of rats

Thus in 1933 when riboflavin was found to have what was called B_2 activity for rats (i.e. it was present in yeast, was heat stable and was needed for growth') it was naturally assumed to be identical both with the rat pellagra and the human-pellagra factors Later research showed that both had to be distinguished from it in turn.

In 1935 it was demonstrated independently by three workers (Professor Paul György Dr Harriette Chick, and the writer of this book) that riboflavin was *not* the rat-pellagra factor Then later in 1935 three of us, Birch György and Harris, jointly proved that this so-called rat-pellagra factor was distinct also from the human-pellagra factor It had in fact been misnamed. Much of this has already been told earlier in the book, but is best sketched in lightly again here for the sake of clarity

The newer name, B_6 was also not in reality very satisfactory because the intervening numbers, B_3 B_4 and B_5 represented nothing more than the rather vague properties of vitamins or mixtures of vitamins which were still awaiting identification So as B_6 prevents dermatitis in rats it was renamed *adermin* signifying a(nti)-derm(atitis)-(vitam)in or later still, *pyridoxin* in allusion to some distinctive features of its chemical structure—it is a pyridine derivative, containing hydroxyl groups—and to its being a vitamin.

SYMPTOMS OF PYRIDOXIN DEFICIENCY

The ailment in rats which we have been discussing is distinguished by a florid dermatitis of the extremities—that is to say there is a very severe inflammation of the skin of the paws nose and ears. The rats may also develop fits—epileptiform seizures. This last-mentioned symptom is found also in pigs suffering from vitamin-B_6 deficiency

It is now known that man needs vitamin B_6 in his diet Its deficiency may be associated with skin troubles resembling those seen in deficiency of riboflavin or nicotinamide as well as with neuritic manifestations.

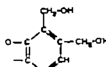
CHEMISTRY OF PYRIDOXIN

A remarkable fact about the chemistry of pyridoxin the rat-pellagra factor is that it resembles nicotinamide the true pellagra factor in being a derivative of the relatively simple substance pyridine



Fig 86 Crystals of vitamin B_6

TABLE LXXVIII *Chemical structure of vitamin B_6 pyridoxin*



(The full chemical name is 2-methyl-3-hydroxy-4,5-di(hydroxymethyl) pyridine)

Pyridoxin was isolated and its structure determined in 1939 and soon afterwards synthesized—those specially responsible being S A Harris and K. Folkers in Rahway U S.A and Kuhn in Heidelberg Germany

The foods richest in vitamin B_6 include yeast, liver pulses and various cereals. One noteworthy fact is that there is ample vitamin B_6 in maize It

follows therefore, that maize, although it is productive of pellagra (p 97) will cure vitamin-B_6 deficiency in animals.

To estimate the amount of this vitamin present in different foodstuffs biological tests on rats on chicks, or on bacteria have been mostly used.

FUNCTIONS OF B_6 IN THE BODY

We know rather less about the chemical role of B_6 than of that of some other B vitamins, such as B_1 or nicotinamide—or of riboflavin discussed in the preceding section It can be said, however that research has shown that there are several substances closely related chemically to pyridoxin which are found to share vitamin-B_6 activity for animals. These are

(1) the parent substance itself —PYRIXODIN
(2) the corresponding *aldehyde*—PYRIDOXAL,
(3) *amine* —PYRIDOXAMIN

Also as a waste-product, excreted in the urine, there occurs

(4) The corresponding *acid* —PYRIDOXIC ACID

It is now known that of these four related substances the actively functioning one seems to be no 2 *pyridoxal* In brief pyridoxal, in the form of its phosphate—pyridoxal phosphate—is concerned in the breakdown of particular constituents of the protein in our food namely the tyrosine, and certain other amino-acids (protein components) The pyridoxal exerts its action in at least two different directions

(*a*) in the *decarboxylation* of the tyrosine and of some other amino-acids (i.e. it acts as what is called an amino-acid co-decarboxylase) and

(*b*) in *transamination* reactions (i e. it acts as co-transaminase)

III. PANTOTHENIC ACID AND CHICK PELLAGRA

Here now is the last of these three additional B_2 vitamins it is the second of the two spurious pellagra-preventing vitamins, and the fourth component of the vitamin-B_2 complex

B_2 (1) NICOTINAMIDE—prevents human pellagra,
(2) RIBOFLAVIN—prevents cheilosis in man
(3) B_6 (PYRIDOXIN)—prevents rat pellagra
(4) PANTOTHENIC ACID—prevents chick pellagra
Other B vitamins—*Folic acid*
Biotin (vitamin H)
Choline
p-Aminobenzoic acid
Inositol
Etc
See Chapter XII

Chick pellagra as its name implies is marked by a severe inflammation of the skin superficially reminiscent of the symptoms seen in human pellagra It is induced by keeping the birds on a diet similar in some respects to that used to produce canine pellagra (blacktongue) It was first studied systematically by Ringrose and his colleagues at Cornell U S A in 1931 For a time the belief was prevalent that chick pellagra was the analogue of true pellagra in humans—i.e due to absence of the same vitamin But then as the chick-pellagra factor was obtained in a more and more purified state—particularly by Elvehjem and his colleagues at Wisconsin—it was found to have the same properties as a substance already known, *pantothenic acid*

PANTOTHENIC ACID AND YEAST

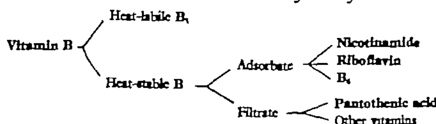
Pantothenic acid was the name given some years previously by R. J Williams of Corvallis, U S A to a substance which as he found, was present in a great variety of different animal and plant materials, and was needed for the growth and vitality of yeast. Its name he derived from Greek *pantothen*, indicating its almost ubiquitous distribution

FILTRATE FACTOR

But pantothenic acid is also sometimes described as a filtrate factor —so this is a third synonym for it. The allusion here is to a different line of inquiry connected with nutritional research on rats. It had been shown that these rats

required some additional B_2 factor distinct from the three already known nicotinamide, riboflavin and vitamin B_6 This fourth component was called the filtrate factor because, unlike the other three mentioned, it was not adsorbed on fuller's earth but instead it passed through into the filtrate from the adsorption This is made clear in Table LXXIX.

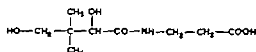
TABLE LXXIX. *Adsorbate and filtrate factors*



PROPERTIES OF PANTOTHENIC ACID

The chemical structure of pantothenic acid was worked out by R. J Williams & R. T Major of Austin, Texas, U S.A. in 1940 It has a relatively simple formula, and is to be classed chemically as a di-peptide derivative (Table LXXX)

TABLE LXXX. *Structure of pantothenic acid*



(The chemical name is α, γ-dihydroxy-β β-dimethyl-butyryl-β'-alanide.)

In poultry deficiency gives rise to the characteristic dermatitis (chick pellagra) already described. In rats there is a failure of growth accompanied by dermatitis, bleeding of the nose the occurrence of a sticky exudate on the eye-lids loss of hair around the nose, greying of the fur and abnormalities in the suprarenal glands Dogs and pigs are among the other species which also need this vitamin.

Pantothenic acid is found in yeast and liver and various other foods. The simplest method for determining it is by a microbe test, similar to that

mentioned for riboflavin (p 265) but chicks or rats may also be used for the purpose

Like other vitamins of the B group pantothenic acid has been found to function in the body as a co-enzyme—and as a very distinctive and remarkable one at that namely one concerned in certain *acetylation* reactions which take place in the living cells. For this reason the enzyme is known as co-enzyme A — A for acetylation This discovery of the biochemical function of pantothenic acid as an acetylating agent was published in 1947 by Lipmann of the University of Texas, Austin U S A (As this edition goes through the press the welcome news comes that Dr Lipmann has been awarded a Nobel Prize in Medicine for 1953 in recognition of this work of his on the acetylation mechanism in living cells which as we now recognize, is of such fundamental importance for our understanding of biochemical events in the body)



Fig 87 Greying of the fur in pantothenic acid deficiency in a rat.

CLINICAL APPLICATIONS

Until recently the possible uses of pantothenic acid in medicine remained obscure Then, in 1946 it was recorded by Dr Gopalan a physician in Southern India, that a deficiency disease common in some areas there, and called—because of its most characteristic symptom— the burning foot syndrome could be successfully treated with pantothenic acid. This was the first definite clinical use for this vitamin.

Somewhat later in 1949 tests were carried out in Britain on patients in hospitals suffering from a special kind of glossitis (sore tongue) which was noted to occur as a secondary symptom in association with certain other disorders (such for example as pernicious anemia and steatorrhoea—these ailments causing in some way an interference in the normal utilization of the nutrients of the diet) In the course of these tests, it was found that the sore

TABLE LXXXI. *Retrospect of Chapter x*

Name of vitamin	Chemical character	Name of corresponding enzyme systems	Biochemical mode of action	Some effects of deficiency		Needed by various types of micro-organisms
				In animals	In man	
Riboflavin	A pyridine derivative	Flavo-proteins	Hydrogen transfer	Rats—growth failure Poultry—curled toe Dogs—yellow liver	Lesions of lips, mouth and tongue	Yes
Vitamin B_6 pyridoxin, pyridoxal, pyridoxamine	" "	Co-decarboxylase	In protein metabolism	Rats—florid dermatitis, fits Pigs—fits	Lesions of lips (?)	Yes
Pantothenic acid	A peptide derivative	Co-enzyme A	Acetylation	Poultry—dermatitis Rats—grey hair bleeding nose, sticky eyelids	Lesions of tongue (?) Burning feet (?)	Yes

tongue could be cured on different occasions by various different vitamins of the B group—but most often of all by pantothenic acid In other words, it appears that a *conditioned deficiency* of pantothenic acid may occur from time to time in hospital practice in Western countries.

This brings us to the end of our account of these three additional B_2 vitamins, the most distinctive feature of which perhaps is that they prevent certain skin diseases, resembling pellagra but yet distinct from it, in various species See Table LXXXI opposite for a general retrospect.

CHAPTER XI

ANTI-ANAEMIA VITAMINS

Folic Acid Vitamin B_{12}

Within the last few years two new and remarkable, vitamins have come to light, and have already proved of great importance in clinical medicine. They are used specifically for the treatment of certain types of anaemia in man These two new vitamins are (1) folic acid and (2) vitamin B_{12}

As folic acid was the first of the two to be recognized, it may be described first.

I FOLIC ACID

One point to note about folic acid, at the outset, is the number of different names under which it had been passing in the early months or years of its history We now recognize that folic acid is identical with (or very closely related to) other factors which had before been severally known as

(1) *vitamin B_c* (preventing a special type of anaemia—hyperchromic macrocytic anaemia—in chicks)

(2) *vitamin M* (needed by monkeys hence called M)

(3) growth-factor for the micro-organism *Lactobacillus casei*

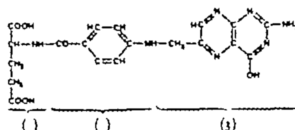
(4) growth-factor for the micro-organism *Streptococcus lactis* R.

Folic acid is present in green leaves and that is the reason for its name (Latin *folium* a leaf) In the rat, in ordinary circumstances, folic acid is being continuously synthesized by bacteria in the alimentary canal, and hence he does not need it in his diet. To precipitate a deficiency in the rat it is therefore necessary to administer a drug to stop the microbes growing—a bacteriostatic agent, of the kind made familiar by the introduction of M and B some years ago (Recall that similar conditions—that is synthesis by microbes in the gut—apply also for vitamin K, pp 250 and 255)

The chemical structure of folic acid was elucidated by a group of American scientists (Angiers and co-workers) in 1945 It is shown in Table LXXXII.

The most remarkable point in the chemist's eye about this structure is the presence in it of what he calls a pterin group It is not surprising therefore that certain other pterin derivatives also have vitamin activity

TABLE LXXXII *Chemical structure of folic acid (pteroyl-glutamic acid) showing how it is built up from simpler molecules*



(1) = glutamic acid.
(2) = p-aminobenzoic acid.
(3) = pteridine derivative
( ) + (3) = pteroyl

FOLIC ACID IN MEDICINE

The great clinical interest of folic acid as Dr T D Spies in America in 1945 and others were the first to show was that when administered in cases of *pernicious anaemia* (and in some other similar types of anaemia—viz. macrocytic megaloblastic anaemias—in man) it was effective in bringing about a regeneration of the red blood cells the deficiency of which is a characteristic feature of the disease However when these investigations were still in the early experimental stages (in 1947–8) a note of caution had to be sounded. It was found that folic acid could not be regarded as a full and complete substitute for liver extract—until then the recognized treatment for pernicious anaemia. Although the new vitamin was successful in bringing about a regeneration of the blood cells it was ineffective in controlling the degeneration in the spinal cord (another ill effect caused by this disease) It might even hasten the development of the degeneration.

Very soon the legitimate uses and the limitations of folic acid in clinical medicine became clearly established. The position can be summarized in a couple of sentences.

(1) Folic acid is a correct, and entirely effective treatment for *certain types* of macrocytic, megaloblastic anaemias, namely the anaemia associated with tropical sprue, and with some other kinds of nutritional macrocytic megaloblastic anaemias [1]

(2) For certain other types of macrocytic, megaloblastic anaemias, particularly for pernicious anaemia, folic acid (at any rate when given by itself) is on the contrary not a permissible remedy since, as indicated above it fails to cure the neurological complications and may even accelerate their appearance. For pernicious anaemia the correct treatment, as will be made clear later (p 279) is the second of these two anti-anaemia vitamins, vitamin B_{12}

FOLIC ACID AND TUMOURS

Another line of investigation but one which at present has to be treated with very great caution indeed, is the possibility of using folic acid or certain folic acid derivatives in the treatment of cancerous growths in animals It was claimed, for example, in 1945 that administration of folic acid caused a regression of spontaneous tumours of the breast in rats However later trials on animals, as well as on man, have given disappointing results Nevertheless, one positive result has emerged. It is now generally agreed that in certain types of leukaemia in man [2] the administration of a particular derivative of folic acid—to be precise, a certain folic-acid antagonist, called aminopterin—can increase the rate of remissions (temporary improvements in the condition) beyond that which could be expected to occur spontaneously without such treatment. The final course of the ailment nevertheless remained unaffected

[1] *Anaemia* is a reduction in the number of red cells in the blood, or in the amount of the iron-containing red pigment, haemoglobin. *Macrocytic anaemia* is an anaemia characterized by an increase in the average size of the red cells of the blood. *Megaloblastic anaemia* is an anaemia characterized by the presence in the blood of megaloblasts (the giant red cells produced by the bone marrow in the process of formation of the normal red blood cells).

[2] *Leukaemia.* This is a disorder in which an unduly large number of white blood cells appear in the blood stream. A characteristic of the ailment, as indicated in the text, is that spontaneous remissions occur from time to time to be followed later by a relapse to the former state and, eventually a general worsening of the patient's condition.

The only conclusion which we can safely draw from these experiences is that the beneficial effect partial and limited as it is, does offer us the hope of possible further developments in the future We can at least say that aminopterin the folic acid antagonist is destined to be a valuable tool in the hands of the research worker

II VITAMIN B_{12}

Vitamin B_{12} is the newest addition to the family of vitamins and in many ways is quite the most remarkable of them all

THE DISCOVERY OF VITAMIN B_{12}

The isolation of vitamin B_{12} came about in an altogether unexpected way At the Maryland Agricultural Experimental Station in U S A. Mrs Mary S Shorb set out to search for a microbe which she could use for the assay by means of a microbiological test, of a particular rat-growth vitamin (the so-called animal-protein factor to be mentioned in the next chapter) Eventually Mrs Shorb found that a certain bacterium called *Lactobacillus lactis* Dorner could in fact be used for the purpose. This microbe, as she ascertained in the course of her investigations, needed two such vitamins, or growth factors The first of the two was present in tomato juice and in some other substances, and the second in liver extracts. The first of the two factors she called the TJ factor (TJ for tomato juice) and the second, the LLD factor (LLD for the name of the *Lactobacillus*) The LLD factor present in liver extracts was shortly afterwards renamed vitamin B_{12}

VITAMIN B_{12} IDENTIFIED AS THE ANTI-PERNICIOUS ANAEMIA FACTOR

A continuation of these investigations by Mrs Shorb with Dr K. Folkers and other workers, soon made it clear that this new factor vitamin B_{12} was identical with the anti-anaemia principle in liver i e, a substance of which there had been some knowledge for many years but which had never yet been isolated in a pure state

THE ANTI-PERNICIOUS ANAEMIA FACTOR

A few words must be interpolated here then about the anti pernicious-anaemia factor as such The name had been familiar to physiologists and medical men ever since some pioneer experiments in 1926 It was in that year that Minot & Murphy in the U S.A. had discovered that pernicious anaemia—a disease which until then had been incurable—could be controlled by the administration to the sufferer of fresh liver or of certain extracts of fresh liver The liver in other words contained some as yet unidentified substance which was needed to regenerate fresh red cells in the blood of the patient ill with pernicious anaemia. And it was this substance, then which was destined to be identified and characterized as vitamin B_{12} in 1947–8

THE ISOLATION OF VITAMIN B_{12}

Another remarkable fact about the discovery of vitamin B_{12} has still to be mentioned. Simultaneously with its isolation by Mrs Shorb and Dr Folkers and their colleagues in America, a second set of scientists, in England, led by Dr Lester Smith at Greenford, Middlesex, had reached the same goal by a different route. Their procedure had been to work up the curative extracts from liver into an ever more and more concentrated form, checking the activity of their product at each stage, not by tests on the growth of microbes but by direct clinical trials upon patients ill with pernicious anaemia.

Eventually Smith and his colleagues obtained the pure substance and this then proved to be identical with the product obtained independently by Mrs Shorb from her very different starting point.

A COBALT-CONTAINING VITAMIN

One unique fact about vitamin B_{12} is that it is the only vitamin so far known that contains a mineral element. The element in question is cobalt. It is the presence of cobalt which accounts for the bright red colour of the vitamin, a feature which had been first noted by Lester Smith and his team during their long and arduous work leading to its isolation.

At this point in our story another fact must be noted in parentheses. This

is that the same mineral element cobalt had previously been recognized as of importance in animal nutrition in another connexion namely as a trace element Its deficiency according to this other set of observations, was the cause of a disease occurring spontaneously among farm animals in various parts of the world—known as pining in Scotland as bush sickness in New Zealand, as coast disease in Southern Australia and enzoötic marasmus in Western Australia—and caused by a shortage of cobalt in the soil, and hence in the pasture upon which the animals grazed

To return to the cobalt-containing vitamin itself

VITAMIN B_{12} IN THE CURE OF PERNICIOUS ANAEMIA

For the treatment of pernicious anaemia in man vitamin B_{12} is usually administered by injection A single extremely minute dose—namely 10 to 100 microgrammes[1]—is found to induce a remission a temporary cure, persisting for some days, or weeks To keep the patient continuously free from symptoms, the injections must be repeated at intervals just as repeated injections of insulin are needed in diabetes A maintenance dose of vitamin B_{12} of 1–3 microgrammes injected daily is found adequate or say 10 microgrammes once every fortnight

Not only does the vitamin B_{12} remedy the disorder in the blood in pernicious anaemia (i e the shortage in the number of red cells, and the consequent low iron content) but there is also apparent an astonishing improvement in the general sense of well being of the patient. Moreover other associated signs, such as the sore tongue are remedied equally effectively and, most important of all the vitamin B_{12} in contrast with folic acid (see p 275) brings about an amelioration also in the nervous manifestations With vitamin B_{12} the disease is entirely under control—if treated in time

In some other varieties of anaemia vitamin B_{12} has also been used with equal success For example in the anaemia associated with tropical sprue vitamin B_{12} —like folic acid—brings about a cure not only of the blood picture but also of the diarrhoea the sore tongue and the general clinical condition.

[1] A microgramme is the thousandth part of a milligramme, or the millionth part of a gramme, and hence 1/28,000,000 of an ounce avoirdupois (cf p 226).

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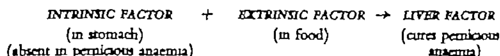
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THE UNDERLYING CAUSE OF PERNICIOUS ANAEMIA

Odd as it may at first sight appear to the reader the bodily defect in a subject suffering from pernicious anaemia in other words the actual cause of his disease, is not any shortage of vitamin B_{12} as such. The defect is rather in the inability of the patient to make proper use of the vitamin from his food. In brief, the underlying cause of pernicious anaemia is that there is a defective secretion of gastric juice and with it of some as yet unidentified substance—an unidentified substance which has been known as the INTRINSIC FACTOR According to the explanation of its mode of action, first advanced by Dr W B Castle as long ago as 1932, the role of the intrinsic factor is to react with some second material present in our food, the EXTRINSIC FACTOR and by so doing to produce for storage in our liver the actual material curative of pernicious anaemia. This was expressed by Castle in the form of an equation



We are now able to say that the hypothetical substance in the food, which was given the name of the extrinsic factor as far back as 1932, is none other than the newly discovered vitamin B_{12}

The explanation now accepted of the mode of action of the intrinsic factor —the substance present in the gastric juice of normal persons but absent in cases of pernicious anaemia—is that it helps to protect the vitamin B_{12} from destruction in the digestive tract (or perhaps it aids its absorption) when the vitamin B_{12} is given by mouth. Knowing then, that patients with pernicious anaemia are short of intrinsic factor with its protective action we can see now why the vitamin B_{12} is most efficiently used when given to them by injection. Because of this absence of intrinsic factor the vitamin B_{12} would be largely inactivated during its passage through the digestive organs if it were given by mouth but if given by injection it is able to short circuit this digestive loss or destruction

A MOULD AS LIVER SUBSTITUTE

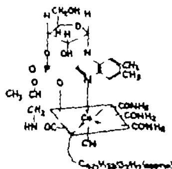
One surprising development has been the discovery that a particular mould named *Streptomyces griseus* contains such large amounts of vitamin B_{12} that it can be used as an alternative raw material in place of liver for the commercial production of this vitamin Its advantage over liver is that much larger quantities of the vitamin can be manufactured from it and at a much lower cost.

OME PROPERTIES OF VITAMIN B_{12}

The chemical structure of vitamin B_{12} has not yet been fully worked out and hence it cannot yet be made synthetically Nevertheless rapid progress is being made in unravelling its constitution and at the time this book is passing through the Press, chemists have already shown that it contains groups possessing the following names 5 6-*dimethylbenzimidazole ribose phosphate cyanide* as well of course as the *cobalt* group The partial formula based on these facts is given in Table LXXXIII

Because of the presence of cobalt vitamin B_{12} has been given the official name cobalamin

TABLE LXXXIII *Partial chemical formula of vitamin* B_{12}



As well as this main form of vitamin B_{12} which contains a *cyanide* group there is another form called vitamin B_{12a} which differs in containing a *hydroxide* group These two forms are denoted by the names cyano-cobalamin and hydroxo-cobalamin respectively

Vitamin B_{12} is sold under various trade labels also such as cytamen or anacobin in Britain and cobione in the U S A

CHAPTER XII

OTHER VITAMINS

In addition to the vitamins already mentioned in Chapters III–XI at least seven other vitamins have come to light during the last few years In most instances the existence of the new vitamin was established as a result of experiments in which animals were fed on some special restricted diet, lacking some component which was thereby proved to be necessary for health.

But, since, with one or two possible exceptions, these newer vitamins are not yet known to be of practical importance in human nutrition, they may be dismissed here with no more than a paragraph or so apiece. For most of them the symptoms of deficiency have been seen only experimentally in animals, and we cannot even say yet whether human beings need these vitamins at all. The probability is that they do since the nutritional physiology of humans and other mammals is in most respects essentially the same.

DEFICIENCIES IN MAN

But even if these vitamins are needed by man it does not necessarily follow that a deficiency is in any one instance likely to occur in actual human experience. That is to say the vitamins may be present in all natural mixed diets, in which case a deficiency would not be likely to arise in practice. This, however is mostly conjecture and it is better to reserve judgement until more is known.

The seven vitamins in question have the following names

Biotin (formerly called vitamin H)	*p*-Aminobenzoic acid
Vitamin F	Inositol
Vitamin P	Strepogenin
Choline	

1 BIOTIN AND THE EGG-WHITE INJURY

The first clue to the existence of a new vitamin later called vitamin H came in 1927 when Miss Boas at the Lister Institute in London found that rats became ill if fed on a special diet containing much uncoagulated (raw) egg-white The symptoms could be cured by cooking the egg white or by making appropriate additions to the diet. Later in 1931 Professor Paul Gyorgy described a factor which he called vitamin H or the *Haut* (German = skin) vitamin It protected rats and other animals from skin troubles—especially from a kind of seborrhoea or greasy and scaly condition of the skin—which occurred when they were fed on the diets again containing the raw egg white It was soon realized that (i) Miss Boas's *anti-egg-white injury factor* was the same as (ii) György's *vitamin H*

Later it was found that it was identical also with

(iii) *Biotin* the name of a growth-factor already known to be necessary for the growth of yeast and other micro-organisms

(iv) *Co-enzyme R*, a growth factor needed by certain micro-organisms functioning in the nodules of peas and other leguminous plants.

The Anti-vitamin in Egg-white

The explanation of the effect of the raw egg white as was shown by Dr R E. Eakin in the U S A in 1940 is that it contains a protein-like substance or anti-vitamin (cf pp 97 189 253) which he called *avidin* this inactivates the vitamin but is destroyed when the egg white is cooked So we now understand why raw egg white induces biotin deficiency but cooked egg-white does not.

Symptoms of deficiency have also been produced in other species for example mice ducks, rabbits, monkeys by feeding them on diets containing raw egg white and lacking the vitamin Dogs develop paralysis monkeys, loss of hair

Whether biotin has any clinical interest is still uncertain One group of American investigators, Dr V P Sydenstricker and colleagues, were able in





Fig 88 Rats suffering from deficiency of biotin.

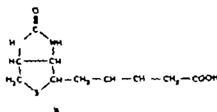
he skin becomes covered with greasy yellow scales—in other words, there is a seborrhoeic
matitis. There occurs also a loss of hair and the rat has an abnormal gait, with a peculiar
ped posture

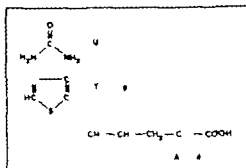
1942 to induce a state of deficiency experimentally in the human subject. This they did by keeping their volunteer on a special diet containing much added raw egg white

One case at least of a deficiency occurring spontaneously in a human being has also been described (by Dr R. H Williams in 1943)

The chemical structure of biotin was elucidated by du Vigneaud and his associates at the University of Cornell in 194.. It can be regarded as built up from molecules of three simpler substances all of interest to biochemists namely urea thiophen and uric acid

TABLE LXXXIV *Chemical structure of biotin showing how it is derived from the simpler substances urea thiophen and valeric acid*





Biotin is found widely distributed in Nature for example in wheat and many seed plants in yeasts and in various animal organs, such as liver kidney etc.

As to its role in the biochemistry of the living animal or plant we now know that it acts as a co-enzyme (cf. pp 69 91 262) controlling certain specific chemical changes that involve a *fixation of carbon dioxide*—in other words biotin is concerned as a co-enzyme in *carboxylation* reactions.

VITAMIN F (ESSENTIAL FATTY ACIDS) AND CAUDAL NECROSIS

1929 and 1930 Drs G O & M M Burr of the University of Minnesota cribed how they had fed rats on a diet which by chemical treatment had n artificially freed—not an easy task—from every trace of fat. The rats eloped a new disease characterized by

a) a scaliness of the tail, sometimes leading to necrosis—parts of the tail ght drop off

b) irregularities in reproductive function

c) abnormalities in the kidney

TABLE LXXXV *Chemical structure of the three nutritionally essential fatty acids linoleic acid linolenic acid and arachidonic acid*

$CH_3[-CH_2]_4-C\ =CH-CH_2-CH=C\ [-CH_2]_7-COO$ LI OLEIC ACID
$CH_3-CH_2-CH=CH-CH_2-CH=CH-CH_2-CH=CH[-CH_2]_7-COOH$ LINOLENIC ACID
$CH_3[-CH_2]_4-CH=C\ -CH_2-CH=CH-CH_2-C\ =CH-CH_2-CH=CH[-CH_2]_3-COOH$ ARAC DON C ACID

urther research established that the cause of the disease was an absence of ain fatty acids. These are characterized by the chemist as being *highly aturated* Thus the work of the Burrs established them as being nutritionally ntial. The names of these *nutritionally essential unsaturated fatty acids* are

1) linoleic acid,

2) linolenic acid,

3) arachidonic acid

Attempts to produce a deficiency of vitamin F in human volunteers have failed. Indeed it is hard to see how a diet entirely devoid of these fats, or fatty acids, could be tolerated by human beings. This being so some people have very reasonably objected to the use of the term vitamin F—even within quotation marks. Let these substances, they argue be denoted by their chemical names as given above

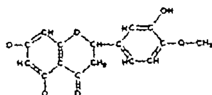
Nevertheless this did not deter advertisers from claiming that vitamin F is the Skin Vitamin and using it in cosmetics. It seems a far cry from caudal necrosis in rats (fed on a diet artificially freed from all traces of fat) to the prevention of facial blemishes in the female film star but sweet are the uses of advertising!

3 VITAMIN P AND CAPILLARY FRAGILITY

Vitamin P is a somewhat controversial issue

Professor A Szent-Gyorgyi with whose name we are so familiar from his celebrated work on vitamin C mentioned in Chapter v described experiments in 1936 which seemed to show that a certain reducing substance (or group of substances) in fruit and vegetable juices (vitamin P citrin or hesperidin) was concerned together with vitamin C in preventing symptoms of scurvy in guinea-pigs. However other workers including the writer and later Szent-Gyorgyi himself had difficulty in confirming this. Vitamin C alone, without vitamin P would cure scurvy in guinea-pigs It did seem however that vitamin P might help to protect or conserve the guinea pig's reserves of vitamin C in a way that certain other reducing agents also are able to Thus vitamin P may be a vitamin-C-sparing substance but possibly no more

TABLE LXXXVI *Chemical formula of hesperitin a form of the so-called vitamin P*



The reason for the discrepancy remains uncertain—perhaps there are some differences in experimental technique still unexplained.

At a later date, it was thought that vitamin P was concerned together with vitamin C in the prevention and cure also of scurvy in humans Especially it was supposed that it controlled the degree of fragility of the capillary blood-vessels In other words the belief was that, in cases of scurvy in human beings, vitamin C sometimes gave only a partial cure and that vitamin P was needed in addition in order to restore the fragility of the capillaries fully to normal, and so to prevent further haemorrhages. Yet other observers including the writer of this book have seen cases of scurvy in man which were undoubtedly cured when pure ascorbic acid alone was given without any additional vitamin P

Because of this uncertainty a Committee in America recommended in 1950 that the use of the term vitamin P should be discontinued.

So there we must leave vitamin P until more facts come to light.

4. CHOLINE, FATTY LIVERS AND METHYL TRANSPORT

There is some philosophical doubt whether choline should be classified as a vitamin or not. For one thing the dose needed by a rat is somewhat large (viz. about 15 milligrammes—1/2000 oz.—as compared say with only 1/200 of a milligramme for vitamin B_1 or 1/40 000 of a milligramme for vitamin D) Besides it can be replaced in the diet by certain other substances, undoubtedly not to be counted as vitamins e.g by *methionine* which is an amino-acid (i.e. a component of protein or main component of the diet, and not an accessory as a vitamin is) or by another substance, called *betaine* (also chemically related to an amino-acid)

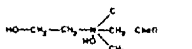
The Chemical Action of Choline

However philosophy is perhaps mostly arguing about words, and whether we call choline a vitamin or not does not matter greatly

The function of choline is of more interest. It is concerned in the body as a kind of chemical reagent, involved in the transfer of *methyl groups*

Table LXXXVII) Chemically speaking, choline is itself the source of these labile methyl groups which it can pass on to other substances needing them Thus it enables the animal organism to make use of an incomplete amino-acid (which lacks the necessary methyl group) in place of the complete one, e.g. methionine (which contains the methyl group) This should be clear from Tables LXXXVII and LXXXVIII

TABLE LXXXVII *Choline and its three labile methyl groups Below betaine which serves a similar function*



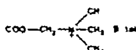
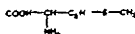


TABLE LXXXVIII *Methionine it also contains a labile methyl group* $—CH_3$ *as marked at asterisk* (cf previous table)



The Symptoms of Deficiency

The effects of deficiency of choline in rats were first discovered in 1934 by Prof C. H Best and his associates in Canada in 1934. This is the same Best who with Sir F G Banting first isolated insulin in 1921 The symptoms of choline deficiency included fatty livers (sometimes leading to cirrhosis of the liver) as well as haemorrhages in the kidney paralysis and other abnormalities.

The explanation of the fatty livers seems to be that the failure in the mobilisation of *methyl* groups mentioned above interferes with the transport of fat—hence too much accumulates in the liver which accordingly suffers damage

From what has been said it will be understood that if a diet contains abundant fat, the need for choline is thereby increased. Conversely if the diet is rich in a protein say casein (which contains much of the amino-acid methionine, already mentioned) the choline can then be dispensed with.

For poultry choline seems to be concerned in the prevention of an ailment known as *perosis* or slipped tendon

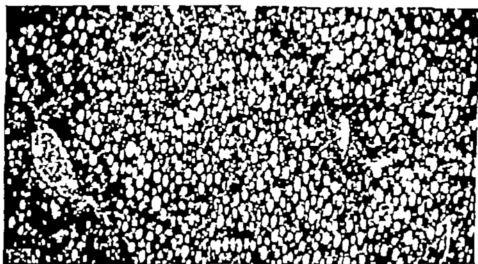


Fig. 89 Fatty liver caused by deficiency of choline.

This is a micro-photograph of a section of the liver, magnified about 90 times.

The hundreds of white spots which are so prominent a feature of the picture, are fat globules, which are seen to have invaded the whole area of the liver and which have distended almost every cell within it.

In human beings, choline deficiency occurring spontaneously is not known but clinical trials have been made using choline as a remedy in conditions in which there is an excessive wastage of protein. (This occurs after burns or wounds or in fevers.) It has thus been recommended for use as a therapeutic substitute for high-protein diets or for methionine, in such cases.

As yet, however choline has no generally accepted use in medical practice.

5 *p*-AMINOBENZOIC ACID (P A B A) GREY HAIRS AND THE SULPHA DRUGS

ll that need be said about the effects of actual deficiency of *p*-aminobenzoic cid (abbreviated to P A B A) is that a greying of the hair has been observed n rats deprived of it and that it seems to be a growth-factor for chickens.

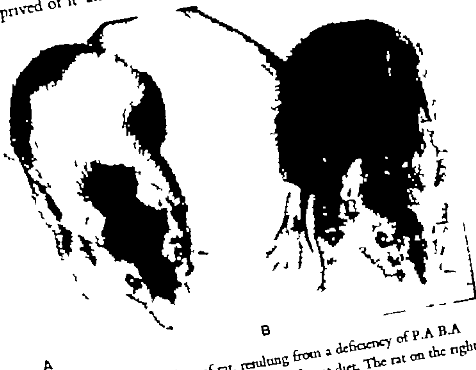


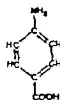
Fig 90 Greying of hair of rat, resulting from a deficiency of P.A B.A The rat on the left had been four weeks on the deficient diet. The rat on the right is one that was cured by administration of P A B A.

P A B A is, however of great importance in the nutrition of many types of bacteria and the real and undoubted interest in it is that it gives the clue to the mode of action of that important new class of drugs, the *sulpha* group —including M and B and other sulphonamides, which have come into such pre-eminence and have saved so many lives in the treatment of certain infectious diseases, including puerperal fever septicaemia meningitis, pneumonia, erysipelas, gonorrhoea and plague.

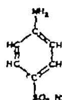
M AND B as Anti-vitamin

The sulpha drug does its job as a bacteriostatic agent (that is preventing the disease-producing bacterium from growing and developing) by depriving it of its supply of this particular essential nutrient, P.A.B.A This was first shown by Dr D D Woods at Oxford. The sulpha is thus an anti vitamin, blocking the supply of P.A.B.A. and preventing its becoming available to the microbe The reason for the blocking is that it has chemically a somewhat similar constitution and thus as it were, competes with the vitamin and gets in its way (Table LXXXIX)

TABLE LXXXIX. *Formulae of P.A B.A. and of sulphanilamide The latter substance is the prototype of the various bacteriostatic drugs (prontosil M and B and other sulphonamide derivatives) which act by inhibiting P.A.B.A.*



p-Aminobenzoic acid



Sulphanilamide

Conversely if extra large doses of the nutrient, P A.B A. are given instead they may negative the bacteriostatic action of the sulpha drug

P.A.B.A. as such does not seem to play any essential role in human nutrition—so far as we are yet able to say It is true that experiments in Egypt and America at one time suggested that large doses of it might exert an anti-infective action in man and animals in some circumstances. Experiences of this kind were reported by observers working on typhus fever in men and mice on Rocky Mountain spotted fever and on certain other experimental infections in guinea-pigs mice and other rodents But, on the whole, the evidence has been conflicting, and experts remain unconvinced that P.A.B.A. itself has any wide practical uses of this kind in combating infections.

6 INOSITOL AND BALDNESS

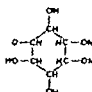
The remarkable thing about inositol is that, if it turns out to be a vitamin for animals (or for some species of animals) as seems likely—then it provides the probably unique example of a substance which can be both a vitamin and an



Fig. 91 Baldness in a mouse caused by a diet deficient in inositol.

anti vitamin For it will be remembered (Chapter VI p 189) that inositol in the form of its hexa-phosphate is the active part of phytic acid, the toxamin which antagonizes the action of vitamin D and increases the severity of rickets

TABLE XC *The chemical formula of inositol*



As to its pro-vitamin properties mice deprived of inositol are said to develop baldness. In rats a similar condition a denuding of the hair around the eyes—picturesquely known as *spectacle eyes*—has been described.

Inositol, like P A.B.A and several other vitamins discussed in this chapter is also a growth factor for numerous micro-organisms, including yeast. As a nutrient for yeast it is one of a group of substances collectively known as Bios (Chapter II) The earlier alternative nomenclatures are given in Table XCI

TABLE XCI. *Nutrients for yeast formerly known as bios*
Scheme of nomenclature

Bios { Bios I = Inositol; Bios II { II A = Pantothenic acid; II B = Biotin } }

One new discovery about inositol has come to light only while this edition was passing through the press. It is this that among the various enzymes present in liver there is one called α-amylase—that is to say a starch-splitting ferment—and quite unexpectedly inositol has been found to enter into its structure. (This was proved by the American biochemists, Lane & Wilson.) We know very little yet about the nutritional significance of this fresh piece of knowledge.

7 STREPOGENIN

Here we reach the last of the newer vitamins to be described in this chapter What is known about strepogenin can be summed up in a few sentences.

Strepogenin is a growth-promoting nutrient—or vitamin-like substance—needed once again, by certain microbes particularly by various *streptococcal* organisms hence its name. It is obtained, as a split-product, from some proteins, e.g insulin in other words strepogenin can be classed chemically as a polypeptide. Other proteins, however e.g egg-white, seem to be devoid of it.

The pioneer research worker on strepogenin was Dr D W Woolley He is noted as one of the foremost biochemists in the U S A and has not permitted the physical handicap of blindness to dim his scientific vision or to cloud his technical skill.

So far relatively little has been done to test the possible nutritive value of strepogenin for animals but it has been said that young rats mice or guinea-pigs kept on some special diets, free from strepogenin, may grow subnormally and can have their growth rates restored to normal when strepogenin is restored to their diets. Nothing at all is yet known about the possible requirements of human beings for strepogenin

CHAPTER XIII

DIETETICS—WHAT TO EAT

I hope that my reader who has borne with me thus far will agree that I was justified at the outset in describing as a fascinating romance this account of human effort and enterprise which has led little by little, to the eventual elucidation of what was once the mystery of the vitamins, to their isolation as pure substances to the discovery of their nature and exact composition, and to the beginnings of our understanding of how they work

DO VITAMINS MATTER?

We must leave now the more theoretical side of our tale and consider its practical applications. What does it all mean to the man in the street? He will no doubt be ready to admit that as a subject for academic research and scientific inquiry vitamins have provided an abundance of interesting—and sometimes thrilling—problems for investigation but how does it affect his daily life? Are his habits of diet open to criticism and if so is it reasonable to expect him to change them? Will he have any benefit to show for it if he does? Surely our ancestors managed all right, before all this new talk of vitamins had been heard of? Apart from which, can he *afford the cost* of the ideal diet? And, after all why not rely on one s natural inborn instinct, or appetite in choosing the right food?

In reply to these questions—and I know from my experience of popular lecturing that these are the posers with which one is always confronted by members of the audience—in reply then I hope in the course of this chapter to convince the reader of the following

(1) That we do in fact have to be educated about nutrition and that instinct alone is not an infallible guide. Primitive man was by no means always well nourished

(2) That an immense amount of avoidable suffering and death in the past has been due demonstrably and unquestionably to an unbalanced diet.

(3) That remarkable improvements in health have been wrought among us, and in our time (and it is not sufficiently appreciated) by better nutrition

(4) And finally—and here is the rub—that it has *already been proved* by large-scale feeding tests on experimental human communities, that when certain improvements and additions were made to our common dietary usage unmistakable benefits followed in physique in stature and in good health and happiness—pointing the way to a still finer sturdier race in the years to come

FOOD SCIENCE I FOOD QUACKERY

Do not imagine that I am asking the reader to become a food crank or extremist or the easy prey of food quacks. It is unfortunately a fact that food science does lend itself very easily to imposition to quackery to crankiness and to specious advertising claims of all kinds. I urge you to beware of these dangers. It may be retorted that the claims that I have just set out myself as to what may be accomplished by nutrition sound equally over-confident. But remember that I am not asking you to accept my conclusions on trust, *ex cathedra* I am about to present the evidence—the bald facts the statistics—and then invite you to draw your own conclusions That is the test which the quacks, the nature healers and the cheap-jacks cannot satisfy

Maybe I shall be criticized by some of my professional colleagues, who hold that research workers should not be popularizers Enough to publish their results in technical form in the appropriate scientific journals! Leave the journalists to their job as best they may! To which criticism I consider it is legitimate to reply that the public demand for knowledge cannot well be denied and that it is better that it should be supplied by those with intimate knowledge than by those whose information is second-hand and less accurate or by those who have an axe to grind, such as by those whose primary interest is in the selling of some commercial foodstuff or preparation

Let us then return to our theme discussing in turn what has already bee[n] accomplished by improved habits of feeding what in our present knowled[ge] constitutes a satisfactory diet, how far in practice we fall short of the id[eal] and finally what practical steps we must take to rectify the errors.

WHAT HAS BEEN ACCOMPLISHED

Recall first that, in the first decade or two of this century rickets of a most severe type was common in our big cities, and as a result large numbers of people suffered the lifelong disability of bow-legs knock-knees and crooked limbs and often crippling for life. To-day severe rickets is rare. This is due in part to a more open-air life, but much more still to the revolutionary *changes in the diet of infants. (For while it is true that enough exposure of* the body to good sunlight can prevent rickets the intensity of the ultra-violet rays of our climate during the winter months is inadequate, and so we have to fall back on the vitamin taken by mouth this is the experience of all the authorities who have tested it out—Chapter VI p 166) The reason why rickets was so common fifty years ago was because babies were brought up too largely on floury substitutes for milk, or on skimmed and evaporated milk with no vitamin-carriers added. To-day we have changed all that, with our insistence on the value of good fresh milk and the necessity of supplementing it with fish-liver oil or vitamin concentrate. This is taught up and down the country at the welfare centres. But much still remains to be done, because we know that to-day no child need have rickets and yet *mild* rickets is still not uncommon (Chapter VI p 164)

Similarly with other vitamin deficiencies. Beri beri, from which so many countless thousands have lost their lives in the East, is now in the list of readily preventable diseases and so is pellagra a disease from which seven thousand people died in the southern states of the *U S A* in one year not so long ago, but which can be prevented with equal certainty (saving the economic anomaly p 74)

Or take another example. For two centuries we have known how to prevent scurvy the disease which ravaged mariners and explorers as well as many isolated communities in Northern Europe (including the British Isles) during the winter months. To this day many people do not sufficiently appreciate the need for an adequate supply of fruit and vegetables in their daily diet.

THE BAD OLD DAYS

We have already dealt with the cynics who protest that we got on all right in the good old days before vitamins were heard of The prevalence of rickets alone is enough to confound them But turn to Fig 92 and Table XCII and consider the truly appalling death-rate of babies in the good old days In 1730, three babies out of every four died before they were five years old. The saving of infant lives is one of the triumphs of modern preventive medicine. We must recognize that it is due largely to improved hygiene, but the work of the welfare centres and the insistence on better feeding have

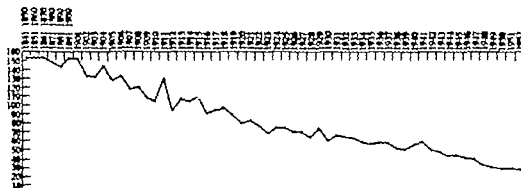


Fig 92 Fall in the infant death-rate

The curve shows the number of deaths of infants under 1 year per 1000 births.

played an essential part too At any rate we can straight away dismiss from our minds as fallacious the appeal to the good old days. As I have said elsewhere it is the argument of that old acquaintance of ours, the old village gossip who ought to know something about babies, her having buried seven

TABLE XCII. *Death-rates*

Out of all babies born in London

In 1730	74%	died before they were five years of age
1750	63%	
1770	50	,, ,,
1833	30%	,,



Fig. 93 Class of elementary school-children at Southwark, 1894. Cf. Fig. 94.



Fig 94 Corresponding class thirty years later Cf Fig. 93

IMPROVED PHYSIQUE

Photographs can be as impressive as statistics Glance at a picture of a group of elementary school children at the beginning of this century (Fig 93) and then for comparison at a corresponding group some twenty or thirty years later (Fig 94) no one can dispute the remarkable change for the better in their physical appearance. If you prefer it in hard figures the statistics of the Ministry of Education afford a striking testimony The change year by year is indeed remarkable. In six years (1921–7) the average weight of children of thirteen at Liverpool had improved by more than 4 lb and their height by more than 1 inch. There was a general and almost steady increase in both height and weight at all ages The same story is told in similar reports from most parts of the country and in Table XCIII and Fig 95 are given some rather more recent returns from another Lancashire town.

TABLE XCIII. *Improved physique of school child*
Average 13-year-old child at Warrington

	Weight				Height	
	Boys		Girls		Boys	Girls
	lb	oz.	lb	oz.	in.	in.
1913	68	5	70	4	53 5	54 8
1933	80	2	87	5	56·6	58·2
1943	86	12	94	8	57 8	58 8

Anyone moving much among adolescents say during the 1930's, was struck by the finer stature of the public school boy compared with the average elementary school boy or even with the grammar school boy of the same age (one saw it convincingly among undergraduates as they came up to Cambridge) Actual tests on elementary school children—and many were carried out—proved how much their physique could be improved and

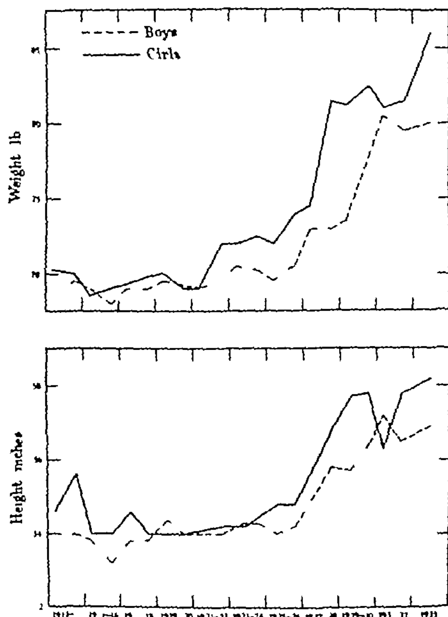


Fig 95 Improved physique of school children. Average weight and height of 13-year-old school children at Warrington, from 1913 to 1933

Note the striking, steady rise during the twelve years from 1921 to 1933 This has continued since see Table XCIII.

brought up towards the public school standard when their diet was suitably added to We shall give the details below Our C3 population in the past has been largely a matter of bad feeding [1]

HOW FAR CAN WE RELY ON INSTINCT?

If, as some would have us believe, we have an inborn instinct which in itself is sufficient guide to tell us what food to eat, there would indeed be no need for professors and lecturers in nutrition and dietetics and infant-feeding at our universities and hospitals. In fact, as A L. Bacharach has playfully suggested, young infants *themselves might most suitably take the place of* the professors, since their natural appetites are presumably the most unspoilt and unsophisticated and they would therefore be able to point out by instinct which foods did the most good. That would be a great saving in trouble and also in expensive salaries!

INSTINCT NOT ENOUGH

But, seriously it is important to realize that instinct was no help to the Japanese *for example, in telling them that they might remain free from the* dreaded plague of beri-beri if they ate their rice with its germ instead of milling it. This had to be found out by research and then driven home by education. And the same story is told by other *deficiency diseases in other parts of the* world. For example, in many regions as in Switzerland, parts of America, and elsewhere the soil and hence the food is deficient in iodide, and this has been the cause of much disease and suffering in the form of *goitre* The Governments have come to the rescue by adding minute amounts of iodide to the drinking water to the salt, or to chocolates given to school children, and with the most remarkable success in stamping out the ailment. *Is it not* idle to maintain that any reliance on instinct could have set right this dietary deficiency? Or again we have already pointed out that in this northern

[1] C3 was the term long used to denote the lowest grade of physical fitness for military service. At the time of the First World War public opinion was shocked to learn how large a proportion of our young men had to be classified as C3

climate of ours most babies are bound to develop some degree of rickets unless given artificial anti-rachitic treatment. Instinct does not come to our aid by telling the babe to demand cod-liver oil! Or to take a final example little of the essential green vegetables and fruits are to be found among our own natural resources in this country during the winter months and the eradication of scurvy in adults, and that later development, the habit of the daily dose of orange juice for all infants with the accompanying eradication of scurvy rickets (Barlow's disease)—a very important achievement—is due not to any natural instinct of man, or to any return to a natural life but simply to science and experience and to education combined with the amenities, or if you prefer to call it so the artificialities of modern transport.

PRIMITIVE MAN

Nor is it right to suppose as some say that dietary-deficiency diseases are a concomitant of civilization only and therefore that all that is necessary is to restrict oneself to natural unspoiled foods Many primitive peoples, e g in different regions of Africa, have been decimated by one dietary-deficiency disease or another depending on their varying tribal dietary customs Numerous instances of this kind can be cited.

INSTINCT OR EXPERIENCE?

We are therefore forced to abandon the old-fashioned view that the right choice of food is a matter that can be settled simply by instinct. It is not an instinct, but rather an art or science which has to be taught. When one comes to consider it, this new view is only in keeping with our changed outlook on other aspects of modern life For example it used to be supposed that simple mother love told the mother all she needed to know about looking after her baby no one needs now to be reminded of the good that has been accomplished by the institution of mothercraft centres, of infant welfare work, of prenatal clinics and such artificial aids to nature Of course intuition has its place but consider how much it can be helped by the long-accumulated experience of the ages (experience being defined as learning from your past

mistakes) and by the findings of modern knowledge. Why even in the most elemental art, that of making love the trend of much modern literature (and a good deal of ancient literature too) *suggests that a certain amount of* instruction is not always or necessarily *de trop* Man learns to imitate the behaviour of the community in which he lives

So much for humans but what of animals?

HOW DO ANIMALS CHOOSE THEIR FOOD

Have animals some infallible instinct, or food conscience, which tells them which foods to choose and which to reject? *Investigations which my colleagues* and I carried out prove that this food instinct or appetite is less infallible than might have been imagined. Under normal conditions of course the natural food found in the district that the animal inhabits, is the right food for it, because, as the principle of natural selection tells us, the suitability of the food is the very reason which has enabled the species to survive in that particular district. Often also there may be a kind of racial tradition and the animal is taught by its parents when young to eat a special food and this becomes a life-long habit. This is certainly often true of human beings

But suppose you offer an animal a choice of all manner of different foods (or food constituents) none complete or self-sufficing in itself but each providing some different essential, or combination of these various essentials. Does the animal thereupon become a dietary expert, and pick out whichever particular food his body is at the moment in special need of? It had often been supposed that animals can choose in this way At the Nutritional Laboratory in Cambridge we carried out a series of tests over a period of years to ascertain how true it was. What we found eventually was that this power of picking out the particular food was possible only under certain rather special circumstances, and by no means always. Putting it very broadly our main conclusion was that animals were able to choose the right food if it made them feel better almost immediately after they had eaten it, and they could then associate their rapid recovery with this particular food which they had just eaten (Figs. 96–99) Expressing it more scientifically and

accurately if a reward follows the eating of a particular food and if there is something about the smell or taste or look of the food by which the animal is able to recognize this food again and distinguish it from other foods he will then learn to select it quite easily That is, it is a matter of experience or trial and error but not instinct.

But unfortunately many things which are necessary in our diet do not have such an immediate effect they do us good but only gradually and they take time to be assimilated into our tissues so that we do not feel any benefit immediately after eating them It is in such cases that it is difficult for the rat to learn that the food is doing him good and therefore he may neglect to eat it although he is continually given the opportunity the food being put before him daily For example no animal is able to survive unless his diet contains a certain amount of protein, and yet if rats are offered pure protein together with carbohydrate fats, etc and allowed to decide for themselves how much to eat, they often have not the sense to take enough protein even to keep them alive and may actually die from protein starvation! The reason it will be understood is that the protein does not produce its effect almost as soon as it is taken in other words does not offer the reward from which the rats may profit for the future

Compare this with some experiments on rats deprived of vitamin B_1 (or B complex) A special feature about vitamin B_1 is that an animal deficient in it is restored to health and activity very rapidly after consuming some of it in particular his appetite returns and his lax muscular tone and alimentary stasis are corrected Hence an animal short of vitamin B_1 and given a little to experiment with soon learns what has done him good and knows to take it for the future The same applies for example also to salt In fact it is known that wild animals in the jungle may travel hundreds of miles for the gratification of a salt lick But an animal suffering from rickets does not learn to take a diet containing cod-liver oil—the reason seems to be that the cure of rickets is slow and you do not feel the benefit immediately you have taken your cod-liver oil (Figs. 96–99)

We have now learnt something about how animals are sometimes able to learn to choose the right food The essential is that *the food must produce a*

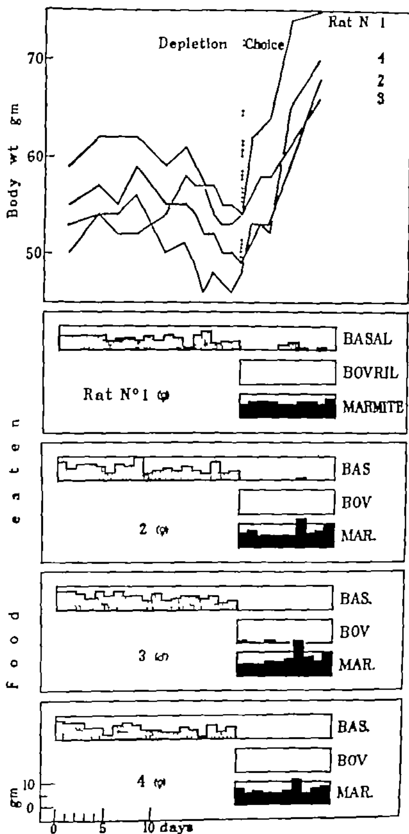


Fig 96. Expt. 1 see p. 312.

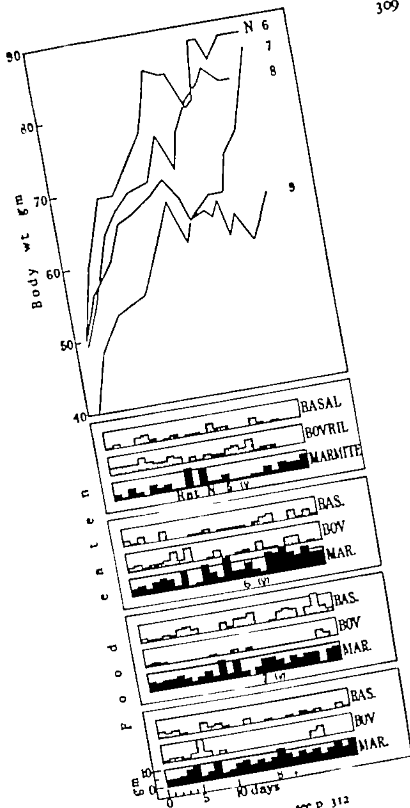


Fig. 97 Expt. 2 see p 312

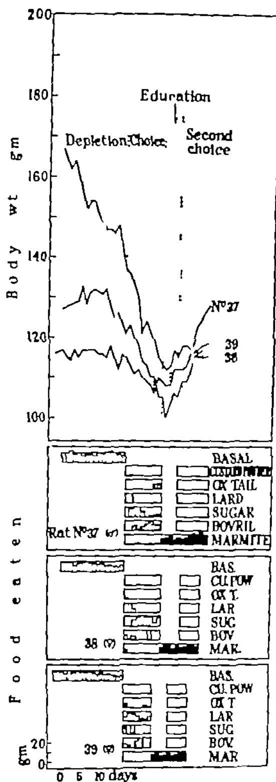


Fig 98 Expt. 3 see p 312

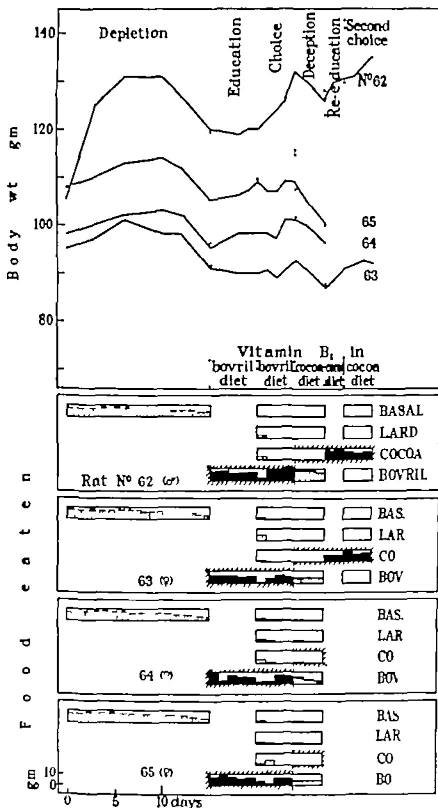


Fig 99 Expt 4 see p 312.

noticeable immediate effect (or reward) which the animal can experience and that the food must possess some distinctive odour or taste or appearance by which the animal can recognize it again next time How does all this apply to human beings?

HUMAN APPETITE

A little reflection shows that the same mechanism is undoubtedly at the basis of much human conduct For example, you demand a hot drink when you are cold because of a subconscious recollection of having felt warmer after you had had one last time A taste for alcohol surely rests on a remembrance (again subconscious maybe) of the pleasant sensations following its previous use—and surely not on any inherited instinct for the supposedly delightful flavour of the beverage The same applies to stimulants such as coffee and tea—a matter of past experience Again the desire for liquid food when you are thirsty is (as I believe) nothing but a subconscious realization that in your previous experience the unpleasant sensations of thirst were relieved when

Figs. 96–99 How rats choose their food. Experiments with vitamin B_1

In each of the bottom panels are shown the various choices offered the rats and the amounts of each they took The food containing the vitamin B_1 is always shown in black, the others are stippled In each of the upper panels are given the growth curves from which it can be seen whether each rat is thriving or not on a given diet

In experiment 1 (Fig 96) rats *in need of vitamin B_1* are found to choose the food which contains the vitamin

In experiment 2 (Fig 97) in contrast rats *not* in need of vitamin B_1 when first offered the same selection show no such preference

In experiment 3 (Fig 98), rats in need of vitamin B_1 were first offered too large a choice and in consequence were too bewildered to be able to discover the right food. But after being *educated* on it for one or two days, and having thus experienced its beneficial effect, they were then able to make the right choice

In experiment 4 (Fig 99) the rats were first [illegible] take the right food, as in experiment 3 Later however they were *deceived* [illegible] *was transferred to a di*[illegible] *food* Under these circumstances the rats continu[illegible] food which had fo[illegible] contained the vitamin and with which their pre[illegible] had been associat[illegible] which now was no longer effective To choose th[illegible] containing the vita[illegible] had to be re-educated [illegible]

Experiments [illegible] show that rats have [illegible] food instinct but able to learn [illegible] i e from rewards [illegible] *Proceedings of the R*[illegible] 3)

you had something to drink Similarly with the elemental feeling of hunger itself The sensation of *bien-être* following a hearty dinner will encourage you to repeat the treatment another time! And again if you have pains after eating dumplings you will soon learn to forgo them (granted ordinary intelligence)

But now compare this with the effects of foods which are less immediate and dramatic in action If a child is getting rickety from insufficient vitamin D or shall we say anaemic from insufficient iron it is hard to see that instinct will teach him which foods would cure him—for the simple reason that they do not produce their good effect quickly enough for him to notice it. But the child will none the less suffer permanent damage from their lack as many thousands do

In point of fact, the average child is inclined to keep to those foods to which he has grown accustomed from an early age—those which he has been brought up on —and he is generally not too ready to experiment with new and strange foods Here is an amusing illustration of this given by Prof. V H Mottram In the good old days the flavour of cod-liver oil was often heartily disliked and a child could be persuaded to take to it only when its flavour was disguised by the pleasanter flavour of jam already well known to him But there are instances of the modern child brought up to like cod-liver oil who when offered jam for the first time can be persuaded to accept it only when it is given a cod-liver oily disguise!

To sum up reason science and human experience must be brought to the aid of appetite in the case of foods which do not act rapidly enough to guide you through the appetite

* * * * * * *

This has been rather a long digression I fear but I hope at any rate that I have now satisfied the reader that in our quest for the perfect diet, it is not sufficient to return to nature or to do as our ancestors did or even to rely on instinct

If you are satisfied so far the next thing to inquire—the crucial question—is, What is the perfect diet, according to the teachings of modern science?

THE ART OF DIETETICS

How can we sum up modern teaching about diet?

I am often confronted with the question Is such-and-such a food better than such-and-such a one? Are oranges more nourishing than brown bread, or oysters than bananas? To such questions I make a habit of replying by asking another question If you were building a house, would you rather have tiles or window panes or floor boards or cement, or plaster or girders, or rafters? The body may be compared with a house and many different foods are needed for building it up and keeping it working properly Some foods provide us with one component and some with another The art of dietetics consists then in choosing a suitable variety of ingredients in suitable proportions.

TABLE XCIV *Food essentials*

Name	Main purpose
Protein	Body building
Carbohydrate, Fat	Fuel
Vitamins	Accessories
Minerals, including calcium, phosphorus, iron, copper iodine, sodium, chloride, potassium, magnesium, cobalt, manganese, zinc, fluoride	
Water	

DIETARY ESSENTIALS AND STANDARDS

To give a list of the various food ingredients which the body needs if it is to remain healthy and survive we must include all those shown in Table XCIV Protein to repair waste and build up new tissues sugar starch and fat to form fuel vitamins to oil the machinery in various ways and a number of mineral salts, are among the most important. Nor must we omit from the list that essential constituent, water

The approximate amounts of some of these nutrients needed in our daily diet are indicated in Table XCV

TABLE XCV *Standards of requirements for nutrients (according to expert committees set up by the League of Nations and other authorities)*

Nutrient	Approximate amount required per day
For normal, average adult	
Vitamin A	3000 international units
Vitamin B_1	300 ,, ,,
Vitamin C	30 milligrammes
Nicotinamide	12 ,,
Riboflavin	1·8 ,,
Calcium	0·5–0·75 gramme
Iron	10 milligrammes
Protein	70 grammes
Total energy value	1000 Calories
For child	
Vitamin D	300 international units
Calcium	1·0 gramme
For pregnant women	
Calcium	1·5 grammes
Iron	> 10 milligrammes

For pregnant and nursing women, and for children, the relative need for most (or all) of these nutrients must be regarded as appreciably increased.

TABLE XCVI *An ideal diet*

Ordinary middle-class diet but must
(*a*) be varied
(*b*) contain daily protein dish (e.g. meat, fish, eggs or cheese)
(*c*) contain daily helping of vegetable or preferably fresh fruit or salad (vitamin C)
(*d*) not be milk-sparing!

With recent conditions of rationing and restrictions in Britain—and, on the other side the provision of vitamin supplements for mothers and babies, the introduction of fortified margarine and national wheatmeal bread and the increased supply of milk in schools—the picture has considerably changed but before the beginning of the Second World War it could be said that in

this country these nutritional needs were roughly met by the conventional kind of middle-class diet, provided it was reasonably varied and contained

(1) at least one good protein dish (meat, fish or cheese or eggs) every day and

(2) a reasonable amount of fresh fruit or salad daily preferably in addition to a vegetable, and

(3) provided that an unfortunate tendency to be economical with milk was checked !

RULES FOR BABY FEEDING

The above applies to adults. The nutritional needs of infants are summarized in Table XCVII

TABLE XCVII *Summary of the ideal infant diet*

Milk basis, supplemented with
1 Vitamin D
2 Vitamin C (orange or black-currant juice, or ascorbic acid)
(3 Iron)

There are three main points to be borne in mind

(1) The basis must be the mother s milk, or a near approximation (e.g a dried milk, or humanized milk etc.)—the old-time floury pastes being held in abhorrence.

(2) All babies in our climate to have additional vitamin D—as fish-liver oil, or synthetic vitamin-D preparation—or to be given a milk preparation already enriched with extra vitamin D otherwise they are almost bound (as all experience shows) to develop at least a mild form of rickets.

(3) All babies to have orange juice daily or preserved orange or black-currant juice, or vitamin-C concentrate as a safeguard against scurvy

A SUMMARY OF COMMON DIETARY FAULTS, PRE-WAR

In the earlier editions of this book, published shortly before the Second World War I stated that some of the most common faults in our national dietary could be summarized under the following headings

(1) *Insufficient protein* This did not apply to the well-to-do—certainly not to anyone who could afford several meat meals a day Their trouble was more likely to be gout[1] But unfortunately there was no doubt that people of the less well-to-do classes were liable to go short of the essential protein element because of its relative expense There was evidence enough that too many growing children in working-class families (and in some middle-class families too) did not get at one meal each day their helping of lean meat, or fish eggs or cheese—which could be taken as the rough guide of a sufficient protein intake which is of such special importance for growing children

(2) *Insufficient milk* Milk too had to be added to the list of important sources of protein in the case of infants and very young children it is obviously the *sole* source or almost so But it also provides other essential nutrients in addition to protein—notably calcium salts. The matter of milk is so essential that we shall return to it again on p 320

(3) *Insufficient vegetables fruit and salad* The well-to-do as well as the poorer classes were sometimes liable to forget the need of a sufficient intake of fresh fruit, salad and of sensibly cooked vegetables.[1]

(4) *Too much* WHITE *bread instead of* WHOLEMEAL, *not enough vitamin* B_1 See below p 327

(5) *Infants and babies not given the necessary supplements of vitamin C (orange juice) vitamin D (cod-liver oil) and iron* See below p 325

(6) *The extra needs of expectant and nursing mothers were too often overlooked* See p 326

EFFECT OF THE WAR

But some of this is now out-of-date For example since 1941 we in Britain have had our national wheatmeal loaf and the pre-war vitamin-deficient, white loaf has been unobtainable.[2] Therefore, no 4 in the above list no longer applies.

[1] Chapter V p. 98

[2] Since August 1953 the sale of white bread has once again been permitted, for those who were hankering after it. Now however several of its known deficiencies are artificially made good, by the compulsory addition of synthetic vitamin B_1 nicotinic acid, and iron. Also, a higher price is charged for this reinforced white loaf which is not subsidized by the government, than for the more naturally nutritious wheatmeal loaf which remains subsidized. This fact acts as some deterrent on the sale of the luxury white loaf.

Let us then break off for a time our discussion of dietary faults, and consider in a little more detail how the war and subsequent development have affected us nutritionally

THE NATIONAL WAR-TIME FOOD POLICY

Perhaps the most urgent problem facing the British Ministry of Food at the outbreak of war was the shortage of shipping space. Since so large a proportion of our food was (and is) imported from overseas every possible cubic yard of shipping space had to be saved It was no longer possible to indulge in the extravagant practice of eating only the white part of the flour ourselves and feeding to farm animals the offal —i.e the bran and the wheat germ so much richer in B vitamins and in other valuable nutrients. Thus it came about that we enjoyed the undoubted blessing of the nutritionally superior wheatmeal loaf, and that in consequence any possibility of deficiency of vitamin B_1 in our restricted war-time diet was abolished.

But equally important was the need for increasing the home production of food Of all home-grown crops potatoes give the most return, acre for acre, in terms of food value. They provide also an important source of vitamin C which is liable to be low when there is a shortage of imported fruits and vegetables.

At the same time every encouragement was given to the cultivation of allotments and people were also instructed in the art of conservative cooking —which means essentially cooking the vegetables in such a way as to conserve their vitamin C (see Rules of Cooking Chapter V p 133)

WAR-TIME SUPPLEMENTS

These were not the only measures taken by a watchful government to eliminate any risks of vitamin deficiencies Some of the others have already been alluded to in passing

Margarine was compulsorily reinforced with vitamins A and D to help make good the loss from butter then in short supply

The special needs of babies (and of expectant and nursing mothers) were particularly safeguarded. On the one hand, preparations containing vitamins

A and D were provided (cod-liver oil or fortified cod-liver oil substitute) and on the other hand—for vitamin C—black-currant purée or concentrated orange juice, or the synthetic vitamin Extra rations were allowed to the mother before and after the birth of her child

SOURCES OF THE VITAMINS IN THE WAR TIME (AND POST-WAR) DIET

It is a remarkable fact that, during the war we in Britain came to rely on a relatively small number of foods from among the many eaten to provide us with the necessary amounts of each of the several vitamins This will be seen from Table XCVIII

TABLE XCVIII *Principal sources of vitamins in war-time*

Food	Important as a main source of vitamin			
	A	B_1	C	D
For adults				
Bread, national loaf		+ +		
Margarine	+			+
Butter	+			+
Vegetables				
Potatoes		+	+	
Greenstuffs	+		+	
Carrots	+ +			
Other roots				
Miscellaneous				
Fruit (when available)			+	
Sugar				
Milk	+			
Meat				
Bacon				
Fish				
Cheese				
Eggs				
For infants				
Vit. C concentrate			+	
Vit D & A concentrate	+			+

For example it will be observed that vitamin A (or its equivalent, carotene) was obtainable in large quantities from carrots and green leafy vegetables apart from these the only other significant supplies came from milk, butter reinforced margarine and the liver oils allowed for babies and mothers.

Vitamin B_1 came from the national wheatmeal loaf, and to a smaller extent from potatoes The amounts in most other foods were relatively small.

For vitamin C we had to rely mainly on potatoes and on green vegetables (especially sprouts and cabbage) and when they were in season, on certain summer fruits notably black currants and strawberries

Vitamin D was present in our reinforced margarine apart from which the only other supply was cod-liver oil, or the other fish oils and concentrates. Hence, let us repeat once again—for it is almost impossible to stress it too often—all babies ought to receive a preparation of this kind otherwise, as all past experience shows, some degree of rickets will be inevitable in an appreciable proportion of them, in a climate such as ours, which is relatively deficient in the ultra-violet rays

Having noted the changes in Britain caused by the war some of which still apply we may conveniently return to a more detailed consideration of common dietary faults.

COMMON FAULTS (1) INSUFFICIENT MILK

The statement that in the past great numbers of children in Britain have received insufficient milk is not so much a matter of opinion as of direct experimental evidence. The evidence is simply this—that children who have been given extra milk have been shown to thrive better than those on a diet otherwise the same but without the addition.

One of the first tests of this kind was carried out in a large orphan asylum in 1926 The diet of the children at this orphanage was considered to be quite good by the then current standards, nor was their general health or their physique below the average.

Dr Corry Mann working on behalf of the Medical Research Council, tested the effect of giving still more milk. The children were already getting

sufficient, many people would have said but the allowance was increased by the addition of an extra pint per day

The effects of the extra milk were indeed remarkable Those having it (one house out of the four in the school) put on weight nearly twice as fast as those not having the extra milk (the other three houses) Compare again the improved growth in height which was about half as much again as in the controls (Table XCIX) In other ways too the physique and health were improved strikingly by this generous abundance of milk.

TABLE XCIX. *Addition of extra milk to institutional diet and its effect on growth and height*
(Corry Mann)

	Average gains per boy in 1 year	
	Height	Weight
Ordinary diet	1 84 in	3 85 lb
,, + casein	1·76	4·01
,, + sugar	1·94	4·93
,, + vegetable margarine	1 83	5 21
,, + watercress	1·70	5 42
,, ,, + butter	2 22	6·30
,, + milk	2 63	6·98

The result was not a matter of chance It was not a case of merely *more food* but specifically of *more milk* Only milk (or the butter derived from it) produced these results. This was proved by giving for example, extra biscuits, or extra sugar etc. in place of extra milk. They produced little or no effect. Various additions were tried but none rivalled milk. Similar tests were carried out in other centres and with the same result.

Every care was taken to make these tests conclusive Those in charge weighing and measuring and noting the health of the children, did not know the details of the experiment or which group a particular child was in so any possibility of unconsciously influencing the result was out of the question! Yet these impartial observers could not help noticing the differences.

v

The children having the liberal extra allowances of milk showed the advantage even in such respects as *more glossy hair* and in the *appearance of their finger nails.* The one black mark against the milk children was that they were more high-spirited and mischievous But perhaps that too is in favour of the milk!

It is related that when one day a representative of the Canadian Government visited the institution to choose suitable strong-looking children to be accepted as immigrants to Canada—and he knew nothing about the milk experiment—it was found after he had finished making his choice that those he had selected nearly all came from the milk group

PRE-EMINENCE OF MILK

Experiments such as these proved, literally with mathematical accuracy that no other food was as good as milk for growing children and that few of them did in practice get enough of it.

Any child who gets less than at least a pint of milk a day is not being given a fair chance.

Since these experiments at the orphan asylum numerous similar tests have been done elsewhere. At council schools at various centres in England and in Scotland, among Maori children in New Zealand at Tokio in Japan as well as in several cities in the United States the effect has been tried of giving children extra milk at school in addition to what they already received at home.

In all instances striking benefits were seen among those children getting the extra milk

SHOULD MILK BE PASTEURIZED?

Something like 7 per cent of the raw milk sold at market in Great Britain was infected with tubercle according to statistics not long since. It was stated that over 2000 fatal cases of tuberculosis in human beings occurred each year caused by such infected milk.[1] The only final remedy would have been to kill

[1] The reader may consult the *Report of the Committee on Cattle Diseases* (*Economic Advisory Council*) 1934, or the writings of Dr A. S Griffith for further information on this subject.

all infected cows, and keep the herds free from infection for the future. But this would have involved great expense and organization—about 30 per cent of cows were infected—and political difficulties intervened. In the meantime the only safe course seems to be to have the milk pasteurized and government action has moved in this direction

It is true that there is some quite minor loss of nutritive value in the milk as a result of pasteurization but surely this is better than the risk of infection! And the loss can be made good, largely at least, by the addition of orange juice (or other vitamin-C carrier) to the babies diet—which should be and is slowly becoming a universal practice

Another possibility is to buy Tuberculin Tested milk which has less risk than ordinary raw milk of carrying infection, since the cows are tested against T.B at intervals, at the source But, pasteurization—either of ordinary or perhaps preferably of T T milk—is the only adequate safeguard. Prices in London in 1953

TABLE C *Cost of milk* 1953

	Maximum retail price per pint, in London	
	June	October
Tuberculin tested, farm bottled	7½*d.*	8½*d*
Tuberculin tested	6½*d.*	7½*d.*
Ordinary (including pasteurized)	6*d.*	7*d.*

SAFE MILK

Although this is possibly rather beside the main theme of this book nevertheless it may be worth while to add a few further comments about this very important topic.

The conclusions given in the foregoing section were first written for the original edition published in 1937 and they have been but little altered for this edition—for they still remain substantially true There has however been some welcome progress in the campaign for clean milk.

Later statistics have continued to show a high toll of life Thus, 1200 deaths from milk-borne tuberculosis were recorded in 1945 according to figures compiled by the Ministry of Health.

An official recommendation that milk should be pasteurized was among the findings reached by a Committee on Tuberculosis in Wartime set up under the auspices of the Medical Research Council. The Ministry of Health has likewise formally advocated the pasteurization of milk as a safeguard against milk-borne disease.

(Milk-borne disease implies, incidentally not only bovine tuberculosis but also other possible infections from the cow and, in addition if to a less degree, illness conveyed from infected persons handling the milk.)

SAFE MILK—NATIONAL POLICY

The *official government policy as defined in 1943 has two aims.* The first aim is gradually to eliminate tuberculosis from the dairy herds of Great Britain. The second aim is to make the milk safe for the consumer particularly by means of pasteurization. Under new sections of the Food and Drugs Act, passed in 1949 and in 1950 the Minister of Food is empowered to denote, progressively areas in Britain in which only specially designated milk may be sold. By such specially designated milk is meant milk which either has been *pasteurized* (or else *sterilized*) *or which has been tuberculin tested.*

It is estimated that, by means of these measures some 48 per cent of the population in England and Wales, or 63 per cent in Scotland, should be safeguarded by 1954.

After this digression about safe milk, we must now return to our nutritional themes.

COMMON FAULTS (2) BABIES NOT GIVEN VITAMIN SUPPLEMENTS

Although since the early days of the Second World War supplements of *vitamin C* (preserved orange or black-currant juice or synthetic C) and of *vitamins D and A* (fish-liver oil or substitute) have by government action been made available for all babies and mothers, it is a regrettable fact, human nature being conservative that many mothers still seem to argue that they don t hold with these new ideas or don t see the need for it their mothers never having used it

It was reported, in a war-time social study that no fewer than 60 per cent of mothers entitled to them did not draw on their vitamin supplements (Chapter VI p 187) The consequence of this lack of enlightenment can be seen in the continued prevalence of mild rickets which could be prevented if all babies had their supplements of vitamin D

In 1944 it was computed, in a nation wide survey that 24,000 babies each year still suffered from rickets (Chapter VI p 164)

COMMON FAULTS (3) IRON DEFICIENCY IN BABIES AND MOTHERS

Three requisites of infant nutrition have now been mentioned (1) the basis of the diet to be milk, (2) a supplement of vitamin D to be given together with (3) a supplement of vitamin C Reference to Table XCVII, p 316, will remind us that there is one further point—namely iron. Now milk is not naturally rich in iron—which the baby must have if he is to avoid anaemia The baby is born with a reasonably good reserve of iron in his liver but this is gradually used up when he is on the milk diet, so that at weaning or before he is likely to be running short.

The problem is solved by using a preparation to which the right amount of iron has been added This may sound a highly artificial procedure yet it is surprising how many babies in the past have been anaemic because their iron was omitted and what really remarkable improvements in general health have resulted when they have had their extra iron Dr Helen Mackay in 1931 found

that in London in the poorer districts no less than 70 per cent of the artificially fed babies, and 42 per cent of the breast-fed, suffered from anaemia. Definite ill-health was caused by the anaemia and there was 50 per cent less general illness when the anaemia was cured. The same condition is not unknown in adults and at the beginning of the present century it was very prevalent indeed among young women In more recent years it has been seen particularly in expectant and nursing mothers in poor districts. For example, some observations in 1935 on working-class mothers in Aberdeen showed that 50 per cent of them suffered from anaemia and consequent below par symptoms. Many of them did not recognize that they were not in good health until after treatment and then they explained that the reason was that really they had almost forgotten what it felt like to be perfectly fit

A word should next be added about the special nutritional needs of mothers

TABLE CI *Iron deficiency (nutritional anaemia) in children and adults*

In children
In London 70 per cent of artificially fed infants
42 per cent of breast-fed infants
were anaemic.
Addition of extra iron to their milk resulted in
50 per cent diminution in morbidity
In adults
50 per cent of women in Aberdeen (poor class) were anaemic.
Cured by iron, with marked improvement in general *health.*

DIETS FOR MOTHERS

Both babies and mothers demand special dietetic consideration. The mother is in need of extra nutriment compared with other adults because of the large amount of food material which she has to pass on to her offspring before birth or in her milk.

In particular she needs plenty of *cow's milk* to provide the extra minerals (especially calcium and phosphate) which are to be taken from her in this way and also extra *vitamin D* to enable her to assimilate these elements effectively

Under war-time rationing there was reason to fear that many mothers sacrificed their own needs for the benefit of their husbands and children Also as mentioned before, not enough mothers took the vitamin supplements provided for them by the Government or through the welfare clinics

COMMON FAULTS (4) DO WE HAVE ENOUGH VITAMIN B_1

In the last edition of this book published just before the Second World War I replied to this question in the following terms

> The answer is generally Yes *provided* we can afford a good varied diet with, for example ample eggs, fruit, vegetables, meat, fish, etc. (Vitamin B_1 you recall is present in reasonable amounts in most such foods as these) But the working class man whose diet consists too largely of white bread and scrape with jam, tea sugar etc. (all devoid of vitamin B_1) and who can eat too little of the more expensive foods just mentioned, is likely to be running low in vitamin B_1 He has therefore in my opinion everything to gain and nothing to lose by scorning white bread and eating instead brown or wholemeal—which contains the vitamin—or a bread which is enriched with extra wheat germ And what we have said of the working class man applies *a fortiori* to the working-class *child*

Government action has since made it easier for all of us to follow this advice, white bread—at least, the vitamin-deficient pre-war white loaf—being no longer available In the interests of sound nutrition it is to be hoped that the new National Bread Policy will be continued.

There is another interesting point to bear in mind about vitamin B_1 Animals short of vitamin B_1 are known to suffer from lack of tone or stasis of the stomach and bowels, as shown in Fig 100. The theory has been advanced that the converse may be true i e that much of the prevalent constipation and stasis in this country has been due to vitamin-B_1 shortage Vitamin B_1 preparations have been accordingly widely recommended for sufferers from constipation. In my opinion the evidence is not yet sufficiently conclusive to

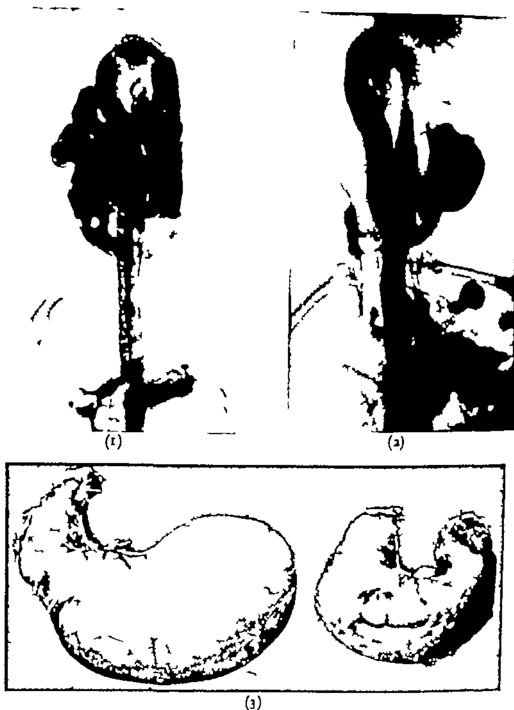


Fig. 100 Intestinal stasis and gastric distension in rat, caused by lack of vitamin B_1

(1) X-ray of colon of vitamin-B_1 deficient rat showing loss of tone.
(2) Normal for comparison.
(3) Distended stomach in vitamin-B_1 deficient rat, on left Normal as control, on right.

show to what extent vitamin-B_1 shortage may have been a major cause of constipation or how often vitamin B_1 is likely to be useful in alleviating the condition—although a number of tests have been made

So much then for adults what of the infant? Does the infant generally get enough vitamin B_1? In America a series of tests, carried out independently by five or six different physicians, showed fairly conclusively that the American babies might be getting sub-optimal amounts of vitamin B_1 (milk is not a pre-eminently rich source of it) and that they gained weight better when extra vitamin B_1 was added to their diet. Not much has yet been tried on these lines in Britain and again we must wait a while before making any too dogmatic statements

COMMON FAULTS (5) TOO LITTLE FRESH FRUIT AND VEGETABLES

There is little doubt that in the past many people in this country had been in the habit of eating insufficient amounts of fresh fruit, salads or vegetables In consequence they had been too near the border-line requirement[1] of vitamin C—*not in danger of getting acute scurvy admittedly which is brought about* by an *almost entire lack* of the vitamin but nevertheless possibly suffering from some *partial* want of it so that their health would have been presumably better for a more ample provision Such people relied too exclusively on potatoes for their vitamin C and in consequence got little more than a bare minimum of it. This is proved by the fact that when there has been a shortage of potatoes definite attacks of acute scurvy have actually broken out among such people. This happened in 1917 in Manchester Newcastle, Glasgow and elsewhere, as mentioned in the chapter on scurvy (Chapter V)

The moral to be drawn is that such risks could be entirely obviated by the consumption of one orange every day or when oranges are scarce, as in war time (and since) by regular provision of greens, or salads or summer fruits in season

[1] The League of Nations standard of requirement is 30 milligrammes per day see Chapter V p. 152.

It is particularly in institutions such as boarding schools, hospitals and boarding houses that the inclusion of fresh fruit vegetables and salads in the menu often tends to be overlooked, and those in charge should give it special attention.

It was surprising too to discover what a large proportion of babies in this country failed to get their daily dose of orange juice, and showed that they

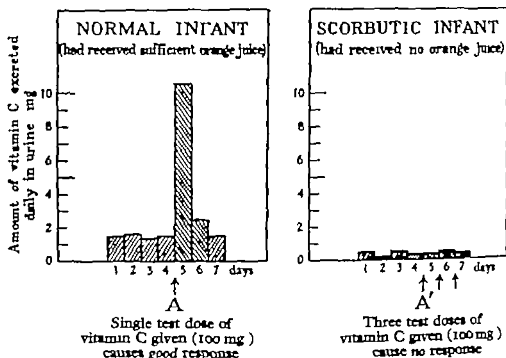


Fig 101 Test to determine if a baby has been receiving ample orange juice.

The amount of vitamin C excreted daily in the urine is measured by means of a chemical test.

On the left, tests on a baby who had received ample orange juice. An appreciable amount of vitamin C is seen to be excreted daily in his urine. At A a large test dose of vitamin C is fed. Since the baby is already well saturated with the vitamin a large amount of it now appears in his urine.

On the right, tests on a baby who had received no orange juice, and was in consequence developing scurvy Little or no vitamin C is being excreted in his urine. When test doses of vitamin C are administered (A') his unsaturated tissues are in such need of it that it is all avidly absorbed with the result that no peak is seen, in contrast with the normal baby until after several days of such test dosing

Similar tests on urine to diagnose a *deficiency of vitamin* B_1 *nicotinamide* etc, have since been worked out (pp 72, 95)

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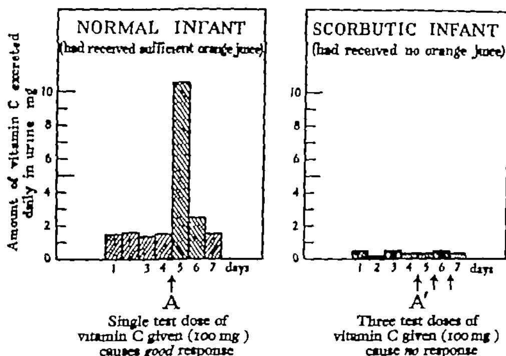


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were at a low level of saturation with the vitamin when we examined them by the test dose method (Fig 101 and see Chapter v p 143)

With Dr Wilfred Fish of the Royal Dental Hospital I investigated the effects of shortage of vitamin C *on the teeth* of guinea-pigs (see Chapter v p 155) There is no doubt that it gives rise to serious faults in tooth structure (such as poor dental enamel and cement) and therefore is a potential cause of decay Now guinea-pigs teeth differ in some respects from humans notably in that they continue to grow (and to be ground away) continuously whereas the tooth of a child is formed once and for all in early life The influence of vitamin C in helping to make sound teeth is therefore of most importance so we believe during the early formative period—i e when the child is still growing Fear of toothache then may be another motive to drive a child—if that is necessary—to eat more fruit

At Chicago Dr Hanke claimed to have had considerable success with that unpleasant trouble pyorrhoea by treating it with very large doses of orange juice (Table CII) The only drawback would appear to be that he found it necessary to give as much as a pint of orange juice per day to bring about a cure smaller doses, say two to three oranges were insufficient. The curative action may not have been due entirely to the vitamin in the orange juice but partly also to other beneficial constituents in it, such as the phosphate We must await confirmatory tests by other investigators.[1]

TABLE CII *Treatment of dental disorders*

(According to claims of M. T Hanke)

Effect of orange juice 1 pint per day	
Caries	50 arrested
Pyorrhoea gingivitis	Mostly cured
Orange juice only 3 oz per day	
Caries	
Gingivitis	Reappeared

[1] Another possible cause of dental decay is shortage of anti-rickets vitamin See below

(An additional reason for eating ample green stuff is that it supplies the necessary vitamin A (in the form of carotene)—without which, as will be recalled the delicate mucous membranes of the body cease functioning normally and tend to dry up and become infected. Vitamin A one gets also from *milk* and *eggs* and *animal fats*, except lard.)

DIET AND TOOTHACHE

It has long been known that rickets is generally accompanied by bad teeth. This was pointed out by Glisson himself in the seventeenth century Hall's striking statistics (Chapter VI, p 16.) on the association of dental caries with rickets at Leeds at the beginning of this century may be cited, as but one example of many similar records which exist.

The explanation as we now know is that the anti-rickets vitamin (vitamin D) *is needed for the proper assimilation of the calcium and phosphorus from* our food. Hence when the vitamin is lacking we run short of these elements and so we cannot lay down properly formed enamel in our teeth—the enamel being the hard outer coat, composed mainly of mineral matter salts of calcium and phosphate. The defect, that is to say is in the external architecture of the teeth. *Hence the teeth being badly protected by this inferior enamel* cannot adequately resist erosion or decay—which means toothache.

May Mellanby supervised a number of large-scale tests on children These showed that when the children were given extra vitamin D the tooth decay tended to spread less rapidly than it would otherwise (Table CIII) The

TABLE CIII *Effect of vitamin D on dental decay*
(From Mrs Mellanby)

	Children given extra vitamin D	Children given no extra vitamin D
Average number of teeth (per child) showing new decay (caries)	1·7	2·6
Average number of teeth in which old decay had spread further	0·2	0·4

vitamin D however did not *entirely prevent* the decay It only prevented it spreading as rapidly as it would have done without it. This indicates that there must be other additional factors making for decay as well as lack of vitamin D Experiments on animals have suggested that relative shortage of phosphate in the food may possibly be one cause. And, as we have also

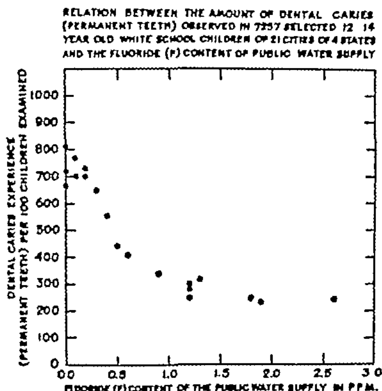


Fig 102. Relation between fluoride intake and prevention of dental caries. In those areas of the United States where the fluoride content of the drinking water is higher the incidence of dental caries is lower

mentioned, insufficient vitamin C is almost certainly another factor which can lead to badly formed teeth and hence to decay But perhaps the most important of all such caries-inducing factors is one which has only recently come to light and that is an inadequate intake of a particular mineral element, namely the trace element, fluorine (Fig 10..)

Dr Evelyn Sprawson of the London Hospital claimed in 1933 that in certain

institutions children who were brought up on *raw milk* (as opposed to pasteurized milk) had perfect teeth and no decay Whether this was due actually to the milk being unheated, or possibly to some other quite different and so far unrecognized cause, we cannot say possibly future investigations will solve the riddle.

THE PROFESSION OF NUTRITIONIST

When this book was first published, in 1935 I wrote

> Disorders of malnutrition as commonly met with in this country to-day are due, not to any fault of scientific knowledge, but either to economic maladjustment—shortage of money—or else to a lack of enlightenment in the principles of dietetics. In some parts of the world active steps are now being taken to combat such ignorance. This is so notably in America, where it has been found worth while to employ whole corps of nutritionists whose function is to advise the public authorities about nutritional conditions and local needs, and to instruct households on the outlay of their budgets. It is to be hoped that like encouragement may be given to the new profession in this country too

It is encouraging to be able to look back, and report, nearly twenty years afterwards, that dieticians and nutritionists have become recognized, accepted and welcomed as a profession in this country and to be able to compliment them on the valuable work they are doing They now have a flourishing professional Association and they publish an excellent Journal.

PARTIAL DEFICIENCIES

Some people still seem to believe that, since scurvy or beri-beri and other vitamin-deficiency diseases are so rarely seen in this country therefore we must all be having enough of the corresponding vitamins (C B_1 etc.)! This is I believe, a fallacious argument. What they overlook is the meaning of a *partial deficiency* By a partial deficiency is meant *some* degree of departure from full health, without any of the more spectacular symptoms of the advanced deficiency disease. In other words we must entirely abandon the old idea that vitamins are merely qualitative factors, that is to say that any trace in the diet, however small, is enough.

On the contrary for any vitamin a certain amount (the so-called *minimum*) is needed to prevent the corresponding deficiency disease but a much larger amount (the marginal dose) is needed to prevent any milder clinical symptoms of illness and a still larger amount (the optimal dose) to ensure full health

This can be mathematically expressed by saying that for any vitamin the curve which relates the response (for example the gain in weight produced

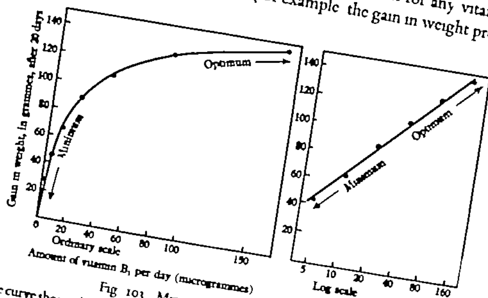


Fig 103 Minimum and optimum nutrition.
The curve shows the improved growth gains in young rats resulting from increased intakes of vitamin B_1 (from data of Waterman and Ammerman, 1935)
See also Fig 25 Chapter III.

in a young animal) to the dose (the amount of vitamin needed to produce it) is approximately logarithmic This perhaps sounds somewhat recondite but is easily understood by a diagram (Fig 103) What it means in words is that we may go on increasing the dose far above the mere minimum needed to prevent the severe deficiency disease and still continue to produce a slight but just perceptible improvement in health physical performance and physique—as can be most readily demonstrated by the weight-gains in a young growing animal.

EXAMPLES OF PARTIAL DEFICIENCY

Two illustrative examples of common partial deficiencies have already been given in this chapter The first was the conclusive demonstration by Dr Helen Mackay and others, that a large proportion of apparently healthy and normal babies and mothers in working-class areas had some degree of nutritional anaemia. Although the babies were not suffering from any obvious illness, yet addition of extra iron did improve their health, as was proved by the subsequent fall in the morbidity rates

The second example is the effect of extra milk. As the reader will know from what has already been said (see pp 320–2) it has now been repeatedly proved with statistical accuracy and all over the world, that the provision of extra milk for supposedly normal —but in reality only average —school children has improved their health, physique and high spirits By the supply of Oslo breakfasts and school meals, and especially the spread of the milk-in-schools habit, the so-called physical class-differences (differences between children of different social classes) are being slowly obliterated.

In other words, we were too long satisfied with the accepted standards What we called normal was only average. Year by year we have seen how *the physique—average weights and heights—of elementary schoolboys have* been steadily creeping up (pp 302–3)

SOME EFFECTS OF PARTIAL DEFICIENCY

We are only slowly learning how to recognize a number of signs by which partial deficiencies of the different vitamins can be detected. An example will make this clear An animal (or human being) might be suffering from some *slight* deficiency of vitamin A—not sufficient to cause the *severe* deficiency marked by the acute eye disease, xerophthalmia—but this slight deficiency could still be detected by showing that the *dark-adaptation* was below standard (p 207) and that it could be cured by vitamin A Another sign of partial deficiency of this vitamin might be that *xerotic* changes (p 236) could be detected in certain membrane tissues of the body

Ill effects due to the partial deficiency of other vitamins, may be listed in a similar way For example a guinea pig (and presumably a human being) may be having enough vitamin C to prevent an attack of definite scurvy but may be having insufficient to promote optimum healing of wounds full resistance to infection or normal tooth-formation during the formative period (p 151)

Similarly partial deficiency of vitamin D even in the absence of rickets may be responsible for an unsatisfactory assimilation of the mineral elements calcium and phosphorus for a flabby muscular tone for minor defects in the bones, and for increased dental caries (p 332)

Again a sub-optimal intake of vitamin B_1 may be the cause of poor intestinal tone or of inferior growth in the young animal, even when the deficiency is not so severe as to cause manifest beri-beri (pp 70 328 335)

ASSESSMENT OF NUTRITIONAL STATUS

It will be clear from what has just been said that the old idea, that the state of nutrition of a child could be at once established by mere cursory inspection by the doctor has to be abandoned. Not so long ago it was customary for the School Medical Service to issue Nutrition Returns in which estimates were made of the extent of malnutrition in different parts of the country These surveys were generally based on nothing more elaborate than a measurement of the child's height and weight, plus an impression of his clinical appearance and the more modern sensitive scientific tests for detecting specific deficiencies were not used at all. It is obvious, therefore that such Nutrition Returns gave us very little information about the occurrence of the milder degrees of deficiency or of the earlier stages of their development.

THE ECONOMICS OF MALNUTRITION

In order to get a clear picture of the nutritional status of our nation, and of our national nutrition problem, we must now turn our attention to a very practical issue that of the cost of a satisfactory diet

It will be simplest, once again, to consider first, conditions as they were at the outbreak of the Second World War because so many special considerations have altered the picture since then—e.g rationing food restrictions labour control and so on.

In the earlier editions of this book it was pointed out that experts were agreed that the lowest price for which a bare sustenance diet could be bought, *just adequate for maintenance of health in an adult man* was from *5s* to *7s* per week in 1934 (or from *7s* to *8s* per week early in 1938) Yet at that time there were large numbers of unemployed and poorly paid workers for whom the expenditure even of the bare minimum was out of the question—and that after paying rent only and allowing nothing for such necessities as coal and clothes.

NUTRITION AND THE UNEMPLOYED

Again in the earlier editions of this book we quoted various surveys which *had been published in support of this statement that large numbers of families* in this country were unable to afford the bare minimum diet.

Thus at Stockton-on-Tees an investigation was carried out by the Medical Officer of Health to determine the money actually available for food in a group of unemployed families chosen at random at a new housing estate. It was found that whereas *5s* per week would have been the amount needed for each man to purchase his minimum adequate diet, the amount actually available was not *5s* but only [illegible]*s.* 10[illegible]*d.* At Newcastle-upon-Tyne in 1934 *the Medical Officer of Health found that one working-class family out of* every three could not afford *any fresh milk at all*—an occasional tin of condensed was all that their means would allow

Similar and equally depressing figures from other districts were available. At Sunderland, for example, the Medical Officer of Health ascertained that the average amount available for food among unemployed applying for assistance in the way of milk, milk foods etc was as low as *3s* [illegible]*d* per person per week. At Southampton an inquiry carried out under an accredited university worker showed that no less than 21 per cent of working-class families were below the poverty line or in other words unable to afford

the absolute necessities of life A similar survey carried out at Merseyside revealed that 16 per cent of working-class families were below the poverty line Or judged by another and rather more humane standard Rowntree's so-called human needs standard 41 per cent of the workers in Southampton and 30 per cent in Merseyside were below the human needs standard —which is defined as the standard below which no class of workers should be forced to live

THE REALITY OF UNDERNUTRITION

We have now reached the stage at which it will be useful to sum up all the evidence which can be collected about the reality the extent, and the severity of undernutrition in this country—that is of the occurrence of ailments, poor health or poor physique due to underfeeding or to faulty feeding

Once again we will start with conditions as they were just before the war i e before the war-time and post war developments introduced so many changes into the picture

TABLE CIV *Evidence of malnutrition summarized* 1937

Type of evidence	Explanation	Main conclusion reached
(1) Economic	Insufficient money available for food, compared with minimum or optimum standards	Poorer classes below standards
(2) Dietetic	Food eaten is inadequate b[y] accepted standards	Insufficient milk, etc consumed
(3) Medical	Existence of disorders of deficiency	Continued prevalence of nutritional anaemia and mild rickets low vitamin-C levels etc
(4) Sociological	Health returns (morbidity physique etc) of ill-fed groups as compared with better-fed groups	Poorer groups at lower health-levels
(5) Experimental	Effect on health in controlled tests when the food is improved	Health improved

a review on the subject which I published in 1937 (*Medical Officer* LVIII 1937 pp 225 237 249 261 273) I said that evidence could be iced along five different lines of approach which I dubbed respectively Table CIV) (1) the *economic*, (2) the *dietetic*, (3) the medical, (4) the ological, and (5) the experimental.

) *The economic evidence* showed that something like 50 per cent of our ulation had insufficient financial resources to be able to afford the diet ed by physiologists to be necessary for full health

) Secondly there was the dietetic evidence by analysis of budgets it been shown that the food eaten was below optimum requirement, notably ie consumption of milk.

) Thirdly there was the medical evidence for example, about 75 per of infants in London slums or 50 per cent of working-class mothers in rdeen had some degree of anaemia 97 per cent of 9-year-old school *lren in L C C schools gave signs of having had some degree (often mild)* ckets 50 to 15 per cent of children were below standard in vitamin-C ves, in the writer s experience

) Fourthly there was the sociological evidence health returns (mor- ty rates, physique) were notoriously lower in poorly fed groups as pared with the better fed in the more prosperous areas

) Fifth and most convincing was the experimental evidence in rolled tests there had been a lowered incidence of infections and better ique and general good health, when the diet was supplemented with e nourishing foodstuffs

ome of the more detailed supporting evidence bearing out the conclusions given under these five headings is tabulated in Table CV Further infor on will be found in the review mentioned at the beginning of this section i such places as Boyd Orr s writings—particularly in his book, *Food, 'th and Income*

TABLE CV Malnutrition 1937 Some of the detailed evidence

Type of evidence	Explanation	Examples
(1) Economic	Insufficient money	About 5 per cent of population below optimum standard (Orr 1936) Unemployed mostly below minimum standard Poverty the main cause of malnutrition (L. of N. Report)
(2) Dietetic	Inadequate food	Milk consumption in Britain 0·2 pints per head per day Cf. parts of U.S.A. N. Europe 1½
(3) Medical	Disorders of deficiency	Nutritional anaemia In infants, 75% incidence in east London (poor areas) In working-class women, 50% incidence in Aberdeen (poor area) Mild rickets Incidence about 50% in children at Woolwich (poor area) Commonest cause of complicated labour in after-life and of crippling Low vitamin-A and vitamin-C status About 30–35% below standard, in test, in poor areas in east London (cf. better-class areas)
(4) Sociological	Comparison of well-fed and ill-fed groups	Nutrition grading At high schools 30% in grade A Among unemployed, 6% Physique Average Glasgow school boy of 13 good social class ¾ stone heavier, 6 inches taller than average boy of poorest social class Infant mortality Doubled, in poor districts, as compared with good districts Morbidity rates Pneumonia and bronchitis, eight times as common in poor district at Newcastle as in good district
(5) Experimental	Effect of better food	Better physique etc. followed, in school children, as result of increased consumption of milk (Corry Mann, 1926 Orr 1928 etc.) Animal test Rats grew better on the better human diets (Orr *et al.* 1935 McCarrison, 1936)

WANT AND PLENTY

In the first edition of this book written in 1933 I said

We may pause to reflect that coincident with this deprivation, food is being burned in many parts of the world, and that in this country fish is thrown back into the sea, or ploughed into the soil The fault here is not with science, but in the existing maladjustment of our social organization. There is still starvation in the midst of plenty The remedies for malnutrition are known, but too often they remain unapplied. So science may be said to be frustrated But if science has been able to achieve so much, discovering how to alleviate human suffering, increasing the productivity of the earth tenfold, need we believe it to be beyond the powers of the human mind to organize and plan our society so that its overflowing wealth and potentialities are made available for man s use?

These views when I first gave expression to them, were still regarded as somewhat heterodox or advanced However they are now quite respectable, and official Already in 1935 Mr Bruce (now Lord Bruce) the Australian Prime Minister while Chairman of the Assembly of the League of Nations, referred to the paradox of glutted markets while insufficient nourishment is available for a substantial proportion of mankind In 1936 the League of Nations Mixed Committee on Nutrition gave their considered verdict that the evidence that inadequacy of diet is widespread is conclusive, the greatest single cause of malnutrition is poverty

ADVANCES IN WAR-TIME

I have alluded repeatedly in the last few pages to the previous (that is, the pre-war) editions of this book, for the reason that it has helped us to make com parisons with the conditions then existing and to draw attention to the undoubted progress of recent years It is a remarkable fact that despite the war—or perhaps it would be truer to say to a large measure because of it—*there* has been a steady advance in the attention bestowed by the public authorities on these important matters of nutrition—and beyond doubt also there has been a corresponding amelioration in the nutritional status of the community taking it as a whole.

Some of the measures taken by the State may well be recounted once again—the substitution of wheatmeal for white bread the vitaminization of margarine the provision of vitamin concentrates and of extra milk for babies and mothers all these have had their beneficial effect But still more important has been the steady growth of the schemes of milk-in-schools, and of school-meals. The physique of the school population in consequence continues to improve and differences of stature and health between social classes are continually narrowing

The introduction of rationing operated in the same direction there is now a fairer share all round and not only for those who can afford it. In other words, a tendency towards a general levelling-up An improving standard of wages lessened unemployment and a feeling for social security have removed the worst examples of those economic evidences of malnutrition referred to above (p 338)

THE FUTURE

Looking back, then over the last few years we can indeed take heart. Our main anxiety must be to see that the advantages gained during the war years and since are maintained.

Let us finally look back a little further still We may recall that during the first quarter of the present century the infant death-rate in England was halved, and the expectation of life greatly increased. Sounder nutrition was undoubtedly one of the factors responsible and had begun to play its vital part in public-health amelioration At the beginning of the century severe crippling rickets was still common In the period between the two wars the knowledge was gained by which rickets could be vanquished. The same was true of other vitamin-deficiency diseases. Thus, during the Second World War measures could be taken with confidence to prevent the risk of the repetition of such occurrences as, when during the First World War there were outbreaks of adult and adolescent rickets e g in Central Europe or of xerophthalmia as in Scandinavia or of scurvy as in Newcastle Manchester and Glasgow or of beri-beri as among our troops in the East

But the prevention of blatant fulminating deficiency diseases is only the first and most obvious step We are now living in the epoch in which nutritional knowledge is ripe to be fully and energetically applied. If buoyant health is the goal of preventive medicine it can only be completely realized when there is, first, the chance of optimal nutrition for all, and secondly a general enlightenment about the means by which it can be attained.

A SUMMARY OF CHAPTER XIII

DIETETICS

Instinct alone is not sufficient.

Diets of primitive races are often far from perfect.

Our ancestors suffered severely from malnutrition.

Remarkable progress has been made but there is still room for further improvement.

* * * * * * * *

For babies special attention should be given to vitamin D fruit juice and adequate iron.

Pregnant and nursing mothers have special need for calcium (as milk) and vitamin D.

* * * * * * * *

Common dietary errors in Britain (and elsewhere) have been

Not enough vegetables, fresh fruit and salad.

Not enough protein for children.

Not enough milk.

Not enough *wheatmeal* bread instead of white.

* * * * * * * *

ECONOMIC CONDITIONS in the past have precluded minimum nutrition for many.

APPENDICES

I THE VITAMINS TABULATED

This table summarizes some of the more important points about those vitamins that are known to be of practical importance for human beings. For summaries of other vitamins and additional information, see pp. 3[illegible], 37[illegible], 2[illegible]2.

CLASS	VITAMIN	CORRESPONDING DEFICIENCY DISEASE	LEADING FEATURE OF DEFICIENCY DISEASE	CHEMICAL NAME (AND FORMULA) OF VITAMIN	MODE OF ACTION OF VITAMIN	PRINCIPAL SOURCES OF VITAMIN
Water-soluble	B_1	Beri-beri ("the disease of rice eaters")	Polyneuritis	Thiamine ($C_{12}H_{17}ON_4SCl, HCl$)	On pyruvic acid	Wheat germ, wholemeal cereals, pulses and nuts, yeast, egg yolk, liver, heart and kidney, pork
	P-P	Pellagra ("the disease of maize eaters")	Dermatitis	Nicotinamide ($C_6H_6ON_2$)	In dehydrogenation	Lean meat, liver, yeast
	C	Scurvy ("the disease of mariners")	Haemorrhages, spongy gums	Ascorbic acid ($C_6H_8O_6$)	On activity of formati[illegible] cell[illegible]	Fresh fruits and salads, vegetables (not over cooked)
	B_{12}	Pernicious anaemia	Macrocytic megaloblastic anaemia	Cyanocobalamin ($C_{63}H_{88}O_{14}N_{14}PCo$) Other cobalamins	Needed for normal maturation of red blood cells	Raw liver, medicinal liver extracts (also prepared from moulds)
Fat-soluble	D	Rickets ("the disease of darkness")	Defective calcified bones	Ergocalciferol (D_2) ($C_{28}H_{44}O$) Cholecalciferol (D_3) ($C_{27}H_{44}O$) Formed from precursors by action of ultra-violet [illegible]	Increases P [illegible] Ca in blood	Fish-liver oil, butter and vitaminized margarine, eggs [illegible] synthetic D_2 [illegible] D_3 from activated provitamin
	A	Xerophthalmia, night-blindness	Membrane[illegible] and slow dark adaptation	Axerophthol (A_1) [illegible]	[illegible]	Liver oils (fish and animal), carrots, green vegetables
	K	Hypoprothrombinaemia	Delayed [illegible] of blood	[illegible]	Prevents [illegible] and [illegible] of [illegible]	Green leaves, fish meal
		Due to [illegible] conditioned [illegible]			[illegible]	

II VITAMINS IN FOODSTUFFS

(For explanation and comments, see p 352)

VITAMIN	FOOD OR PREPARATION	VITAMIN ACTIVITY (in terms of milligrammes or international units per 100 grammes of food, uncooked or as purchased)
Fat-soluble group		
Vitamin A	(i) Halibut-liver oil	3 000,000–15,000,000 I.U.
	(i) Cod-liver oil	50,000–200,000
	(i) Liver calf or ox	5,000–15,000
	(ii) Butter	2,000–5 000
	(ii) Margarine, vitaminized	2,000
	(iii) Red-palm oil	50,000–200,000
	(iii) Carrot	10,000–20 000
	(iii) Spinach green-leaf vegetables	5 000–20,000
Vitamin D	Tuna-liver oil	2,000,000–6,000,000 I.U.
	Halibut-liver oil	100,000–300,000
	Cod-liver oil	10,000–30,000
	Herring-body oil	10,000–20 000
	Cacao-shell oil	30,000
	Egg-yolk	200–400
	Margarine, vitaminized	200
	Butter	30–100
Vitamin E	Wheat-germ oil	250 mg
	Rice-germ oil	100
	Cotton-seed oil	100
	Green leaves	5
Vitamin-B complex		
Vitamin B_1	Dried brewer's yeast edible food yeast	1 000–2,000 I.U.
	Barley germ	1 500
	Wheat germ	500–1,000
	Rice bran	500
	Oatmeal	100–200
	Wheat, whole grain	100–200
	Wholemeal bread	100
	Peas	100
	Haricot beans	100
	Egg yolk	100
	Bread, national loaf	60

(*Continued on next page*)

VITAMINS IN FOODSTUFFS (*cont.*)

VITAMIN	FOOD OR PREPARATION	VITAMIN ACTIVITY (in terms of milligrammes or international units per 100 grammes of food uncooked or as purchased)
Vitamin-B complex (*cont.*)		
Nicotinamide	Yeast, dried	5 50 mg
	Liver	15 0
	Peanuts	10–15
	Salmon canned	[illegible]
	Meat (beef liver pork)	5
	Wheat, whole	5
	Egg, whole	0·1
	Milk	0·1
Pyridoxin (B_6)	Yeast dried	4 mg
	Wheat germ	1
	Molasses	0 3
	Liver	0
	Fish muscle	0 1
Riboflavin	Yeast, dried	[illegible]
	Liver	3
	Kidney	[illegible]
	Egg	0·3–0 4
	Milk	0·1–0
Vitamin C	Black currants	150– mg
	Paprika	150– 00
	Kale	1 0–140
	Brussel sprouts	75 125
	Cabbage cauliflower	50–100
	Strawberries	60–80
	Spinach	50–70
	Orange juice	50–70
	Lemon-juice	40–60
	Grapefruit	35 45
	New potatoes	30–40
	Tomato-juice green peas	20–30

(*Continued on next page*)

Notes on Appendix II (Vitamins in Foodstuffs)

The object in the foregoing table has been to include the richer sources of some of the more important vitamins only For the convenience of the reader certain values that have already been given in the body of the book (e.g pp 57 130) have been re-assembled here.

In using this table several facts may be borne in mind

(1) *Variability* Vitamin activity can vary appreciably from one specimen of a food to another and the figures given in this table are to be regarded as no more than typical of an average range of values To illustrate this, the entries given in this table are of the same order of magnitude as, but not necessarily precisely identical in all instances with, those in Tables XIX and XLI.

(2) *Vitamin requirements* The reputed daily requirements, as already mentioned on p 315 and elsewhere, are as follows vitamin A, 3000 i.u. vitamin D (prophylactic, for child), 500 i.u. vitamin B_1 3000 i.u. nicotinamide, 12 mg riboflavin, 1 8 mg vitamin C, 30 mg

(3) *Units.* For calculating the vitamin contents of diets, it is useful to know that 100 grammes (in the scientific, metric, or continental system of weights) are equal to 3 5 ounces or 0·22 lb (in the avoirdupois English system)

(4) *Losses on cooking and processing* The vitamins most liable to be affected by cooking, or by processing (e.g canning) storage or ageing, are C and B_1 The amount lost can depend on various circumstances (pp 54, 131 and 134)

(5) *Vitamin A.* Vitamin A is more completely utilized than is carotene. Foods grouped in this table under heading (i) contain vitamin A only those under (ii) carotene plus vitamin A, and under (iii) carotene only

(6) *Vitamins in practical dietetics* Foods of special consequence as vitamin carriers in the British national diet (especially in times of war or shortage) are mentioned in Table XCVIII, p 319 Dietetic teaching is summarized at the end of Chapter XIII.

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